STUDIES OF THE BETA HEMOLYTIC STREPTOCOCCUS (SMITH AND BROWN)*

WILSON GEORGE SMILLIE

From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston

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INTRODUCTION

Septic sore throat, or epidemic tonsillitis, is an acute, severe, communicable disease prevalent in the United States. It has not been clearly separated from sporadic tonsillitis, and in some instances has been confused with scarlet fever. The physician has no definite clinical or laboratory criteria on which to base a diagnosis. If a case of tonsillitis is severe, with high fever, prostration, ulceration of, and exudate on, the tonsils, and angina, the conscientious physician properly reports the case as septic sore throat; other physicians do not report such a case unless the disease is present in the community in epidemic proportions. In the mind of the health officer septic sore throat, when epidemic, is a disease due to the use of contaminated milk. There is so much confusion about the disease that any facts which tend to clarify the problem of its etiology, and more particularly the mode of its transmission, are of scientific interest and practical importance.

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HISTORICAL REVIEW

Severe inflammations of the throat due to the use of contaminated milk have been recognized in England[1] for more than 30 years. In this country, before the year 1908 no epidemics of tonsillitis due to the drinking of contaminated milk had been reported. Following the epidemiologic studies of the Boston epidemic by Winslow,[2] however, at least two epidemics of septic sore throat each year have been reported as due to the use of contaminated milk or milk products.

The most extensive studies of this disease are those of the Boston epidemic by Winslow; of the Chicago epidemic in 1912 by Capps and Miller,[3] and Davis[4] and others; and of the Baltimore epidemic by Frost,[5] and Hamburger.[6] Streptococci from the first have been believed to be the etiologic factor. Careful study of the characteristics of the streptococci isolated has been made in only a few instances.

Rosenow[7] in 1912 carefully studied the streptococcus from the Chicago epidemic. He described it as (1) virulent for rabbits, (2) growing abundantly in ordinary media, (3) encapsulated, (4) without long chains, and (5) with but little hemolysis. His experiments tended to show that pyogenic streptococci, when placed in unheated milk, were transformed into the type found in septic sore throat. In a publication of the same year Davis and Rosenow,[8] describing the streptococcus from the Chicago epidemic in greater detail, differentiated the streptococcus of septic sore throat from Streptococcus pyogenes, or the common hemolytic streptococcus, by (1) the relatively narrow zone of hemolysis, beginning immediately around the colony, with an outer indistinct zone, (2) the presence of a capsular substance, (3) the nonfermentation of inulin, and (4) virulence for animals. They suggested that it lay midway between Streptococcus pyogenes and Streptococcus mucosus. Davis[4] added to these characteristics the fermentation of dextrose, lactose, maltose, saccharose, and dextrin, and the nonfermentation of mannite, raffinose, and inulin.

Hamburger[6] described the etiologic agent of the Baltimore epidemic as a short-chained streptococcus with a "halo," which in media tends to form long chains. Blood agar showed a hemolytic zone. Inulin was not fermented.

Theobald Smith and J. Howard Brown[9] (1915), in studies of streptococci isolated from presumably milk-borne epidemics of tonsillitis in Massachusetts (1913-1914), found certain definite characteristics: (1) A definite type of hemolysis (called by them the "β type of hemolysis"), which gave a sharply defined, clear, transparent, completely hemolyzed, colorless zone from 2 to 4 mm. in diameter around the colony. Under the microscope no corpuscles were seen within the zones. The colonies were simple or biconvex, never complex. (2) Fermentation of salicin, nonfermentation of raffinose and inulin. (3) Characteristic virulence for rabbits.

In further studies of streptococci isolated from the Boston, Baltimore, and Chicago epidemics, Smith and Brown found in each instance a streptococcus

[1] Bacteriology of Milk, 1903.
[2] Jour. Infect. Dis., 1912, 10, p. 72.
[3] Jour. Am. Med. Assn., 1912, 58, p. 1848.
[4] Ibid., p. 1852.
[5] Bull. 5, Hyg. Lab., Washington, D. C., p. 1889.
[6] Jour. Am. Med. Assn., 1912, 58, p. 1109.
[7] Jour. Infect. Dis., 1912, 11, p. 338.
[8] Jour. Am. Med. Assn., 1912, 58, p. 773.
[9] Jour. Med. Research, 1914, 31, p. 455.

of this same type. All the types isolated did not have identical carbohydrate reactions; one group did not ferment lactose, and one group fermented mannite, tho most of the groups did not. Smith and Brown accorded no emphasis to capsule-formation or length of chain as constant or significant.

An important point brought out by Smith and Brown was that these streptococci were, they believed, of human origin with characteristics readily differentiable from those of bovine strains. Septic sore throat, they held, is not due to a bovine strain of streptococci from a "gargety" cow. Udders of cows become infected with human streptococci, which are only mildly pathogenic for cattle, but which may remain in the udder reservoir for some time, multiplying and heavily contaminating the milk. In Outbreak B, Smith and Brown isolated from a cow a streptococcus which had characteristics similar to those of the human strains of the same epidemic.

These observations were substantiated by the studies of Krumwiede and Valentine,[10] who isolated a streptococcus from the udder of a cow in a dairy from which infective milk had come, which in its characteristics was similar to the beta hemolytic type. Inflammation of the cow's udder was slight. Agglutination tests further identified the streptococci from the throats of patients with those from the udder of the cow. One dairy worker had a sore throat, but the authors were unable to isolate the beta hemolytic streptococcus from him.

The probable method of infection of the cow's udder with a human strain of streptococcus is demonstrated in the experiments of Davis and Capps.[11] They infected a small abrasion on the skin of the teat with streptococci from tonsillitis and septic sore throat; slight redness and tenderness resulted, and the human strains of streptococci were present in the milk at the end of 4 weeks. A like result followed injection of a culture of a human strain directly into the duct. The streptococci did not lose their characteristics during the 4 weeks in the udder of the cow.

The essential characteristics which, according to Smith and Brown, distinguish this streptococcus as a human strain have each received consideration by other investigators.

Carbohydrate Reactions.—The best recent work on the carbohydrate reactions is that by Broadhurst,[12] which includes a review of the literature together with a study of over 700 strains of streptococci. She concludes that it is impossible to differentiate human from bovine strains by means of the carbohydrate reactions.

Hemolysis.—The results of study of streptococci from the standpoint of hemolysis have been unsatisfactory because of the lack of a standard technic. Streptococcus pyogenes has been generally recognized as hemolytic, but without any clear definition of the type of its hemolysis. Different workers have used the blood of different animals, mixing the blood with agar prepared in various ways, and in varying amounts, and reading the hemolytic plates at varying times after incubation. Comparative results are therefore almost without value.

Ruediger,[13] as early as 1912, suggested a differentiation of S. pyogenes in milk from S. lactis by the fact that S. pyogenes produces small colonies in blood agar, surrounded by a large zone of hemolysis, while S. lactis produces

[10] Jour. Med. Research, 1915, 28, p. 231.
[11] Jour. Infect. Dis., 1914, 15, p. 135.
[12] Ibid., 1915, 17, p. 277.
[13] Science, 1912, 35, p. 223.

grayish or greenish colonies, with little or no hemolysis. Puppel[14] brought out the same facts. Later studies have shown that hemolytic streptococci may be found in bovine feces (Broadhurst[12]) and thence in milk. It has also been shown that all hemolytic streptococci in milk are not pathogenic for rabbits. Hemolysis alone is therefore not sufficient basis for differentiating human from bovine strains.

Virulence for Animals.—Virulence for animals as a basis for the classification of streptococci, has received less attention than carbohydrate-fermentation and hemolysis. Rosenow,[7] in an epidemic of septic sore throat, isolated a streptococcus with characteristic cultural properties from both the milk and the patients by intravenous injection of a suspension of the suspected material into rabbits; a characteristic polyarthritis was produced in the rabbits, from which pure cultures of hemolytic streptococci were obtained. He did not think it possible to differentiate human from bovine strains of streptococci, because the streptococci were so modified in the milk as to be transformed into the types found in septic sore throat. Jackson[15] also obtained a characteristic polyarthritis in the rabbits by injection of streptococci from the Chicago milk epidemic.

I have had the opportunity to repeat the work of Smith and Brown in 2 milk-borne epidemics of septic sore throat. These were studied from both epidemiologic and bacteriologic points of view. In each epidemic a strain of streptococci was isolated from the throats of the patients and from the milk, and in one case from the udder of the cow, which corresponds in all its characteristics with the type of streptococcus reported by Smith and Brown.

Since the beta hemolytic streptococcus appears to be a human strain, it must follow that milk can be contaminated only from some human source — directly by handling, or indirectly through infection of the cow's udder. This being the case, it is of importance to know the prevalence of this strain of streptococci in human throats, the discharges from which are the most widely disseminated of all human excreta. Is the beta hemolytic streptococcus present in normal throats? If so, the discharges from the mouths of all of us are a potential source of danger. Is the beta hemolytic streptococcus found in the throats of individuals after recovery from septic sore throat, and how long is the individual, in such case, a source of danger to the community? Does septic sore throat occur sporadically, or only in epidemic form? If sporadically, what proportion of ordinary tonsillitis is due to the beta hemolytic streptococcus? Finally, what relation has the beta hemolytic streptococcus to scarlet fever?

In answer to these questions I submit the results of the study of 100 cultures from normal throats, and of cultures from the throats

[14] Ztschr. f. Hyg. u. Infektionskrankh., 1912, 71, p. 3.
[15] Jour. Infect. Dis., 1913, 12, p. 364.

of 20 individuals who have had tonsillitis in a definitely milk-borne epidemic within the past 2 years. A contact epidemic of septic sore throat due to the beta hemolytic streptococcus also is reported because it seems to illustrate the connection between sporadic and epidemic occurrences of the disease. The presence of the beta hemolytic streptococcus in the throats of 20 patients with typical follicular tonsillitis is noted and also the results of the study of cultures from the throats of patients suffering from various types of scarlet fever.

THE CAUSATIVE AGENT OF THE DORCHESTER MILK-BORNE EPIDEMIC OF SEPTIC SORE THROAT, 1915

On April 23, 1915, an epidemic of sore throat was reported to the Boston board of health from the suburb of Dorchester. There had been no sporadic cases reported previous to this date, nor had there been undue incidence of tonsillitis. The epidemic, which lasted for 6 or 7 days, was practically limited to Dorchester.

EPIDEMIOLOGIC STUDIES

During the week of April 23 to May 1, 295 cases of septic sore throat were reported in the Dorchester district. Of these, 57 were not typical clinically, 5 were definitely diagnosed as scarlet fever, and 6 were not found by the health officer. The remaining 227 cases were typical of septic sore throat.

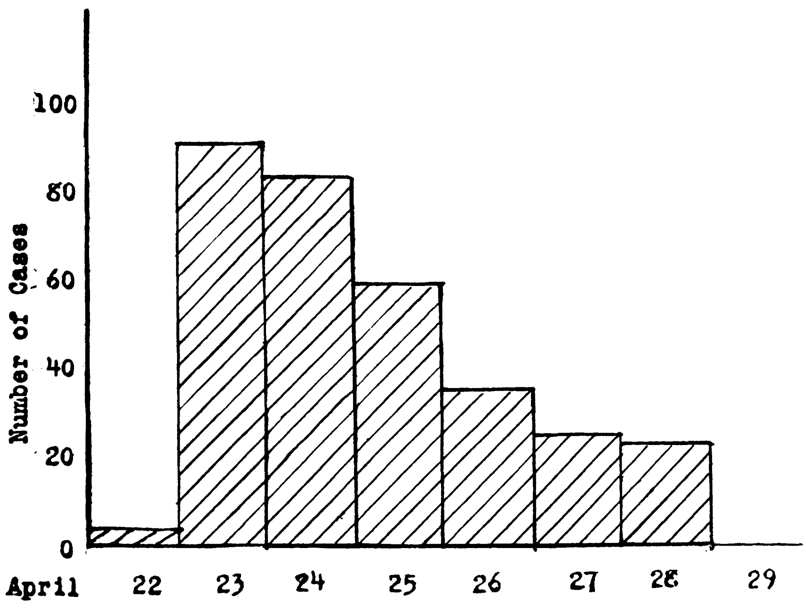

Chart 1. Case incidence of the Dorchester epidemic of septic sore throat.

The curve of case incidence, shown in Chart 1, is rather typical of a milk-borne epidemic. This curve is really a record of the cases as reported to the board of health, but since most of the cases were reported on the first day of the disease it closely portrays the true case incidence of the outbreak.

There was a variation in the virulence of the disease: 50% of the patients were only slightly ill; 40% were moderately ill; and 10% were seriously ill, two deaths being directly attributable to the acute disease. Several succumbed to a resulting pneumonia or to other complications, of which endocarditis and otitis media were the most common. There were no cases of peritonitis.

An investigation of the milk supply of the 227 typical patients gave the following results:

Oak Grove milk, pasteurized	5 cases
Manning's milk, not pasteurized	12 cases
Pond's milk, not pasteurized	203 cases
Codman's milk, pasteurized	5 cases
Gushee's milk, not pasteurized	1 case
Moore's milk, not pasteurized	1 case
Garrity's milk, not pasteurized	1 case
McShane's milk, not pasteurized	1 case
Redmond's milk, not pasteurized	1 case

The conspicuous Pond dairy is a comparatively small one, supplying only a small proportion of the milk used in Dorchester, but almost 90% of the typical cases were on Pond's milk route. Pond supplied milk to Dorchester only, and the epidemic was limited to Dorchester. It seems most probable, therefore, that Pond's milk was the cause of the epidemic.

This dairy was the distributing station of 3 small farms (C. F. and B.), Pond owning the milk route, the wagons, the bottles, etc. There had been no illness among any of the farmers except in the case of one lad (A. B.) of 19 years, who 3 weeks previously had had sore throat and fever. No rash had been noted, but at the time of visit the health officer noted a characteristic desquamation of the hands and feet. The boy had received practically no medical attention, had been in bed but a few days, and was at the time of visit in good health. During his convalescence he had been helping with the milking. The board of health considered it probable that this boy had had scarlet fever, and had been most likely the source of the contamination of the milk.

Bacteriologic Studies

On April 26, throat cultures were obtained from swabs collected by Dr. Ceconi, the district health officer, from all those who were connected with the preparation of Pond's milk for the market, and from typical cases of the disease. A sample of Pond's milk, taken April 23, was also obtained for study.

The throat swabs were at once placed in 10 c.c. of normal salt solution. One small platinum loop (2 mm. in diameter) of this suspension was added to 10 c.c. of meat infusion agar containing 1 c.c. of horse blood. Plates were then poured and incubated. At the end of 24 and 48 hours the plates were studied, and colonies suggestive of the beta type of hemolysis could be isolated. In case the colonies on the plate were too numerous, duplicate plates were made with one-tenth the first amount of suspension (the normal salt suspension being kept in the ice box for just this emergency).

Table 1 gives a summary of the clinical observations and preliminary laboratory findings. The throats of all the patients, the sample of milk, and

the throats of 5 of the dairymen yielded streptococci of the beta hemolytic type. Those patients who were acutely ill gave larger numbers than those who were moderately ill. Once or twice the beta type was found in a throat which to all clinical appearances was normal.

In order to determine whether the hemolytic streptococci found in the throats of the patients and the dairymen, and in the milk from Pond's dairy, were identical, further cultural studies were undertaken. Typical colonies of

TABLE 1

CLINICAL AND PRELIMINARY LABORATORY OBSERVATIONS IN THE DORCHESTER MILK-BORNE EPIDEMIC

Source of the Strain	Clinical Condition		Beta Hemolytic Streptococci from Throat
	General	Local	
Milker on C. farm	Excellent	Throat apparently normal	None
Milker on C. farm	Excellent	Throat slightly congested	None
Worker on C. farm	Not prostrated; at work in dairy	Throat deeply congested	100 clear-cut colonies in almost pure growth
Worker on F. dairy	Prostration. Fever 102	Deep injection of throat, ulceration of and exudate on tonsils	Large numbers
Worker on F. farm	Excellent	Throat apparently normal	8-10 colonies
Worker on F. farm	Excellent	Throat apparently normal	8-10 colonies
Worker on F. farm	Excellent	Throat apparently normal	None
Worker on F. farm	Excellent	Throat apparently normal	None
Milker on B. farm	No acute disease; general condition poor	Chronic inflammation of throat	None
Worker on B. farm	No prostration, no fever	Throat injected and slightly painful	None
Worker on B. dairy	No prostration, no fever	Throat slightly injected, profuse nasal discharge	Almost pure growth
Worker on B. dairy	Desquamation of hands and feet	Throat apparently normal. Acute sore throat and fever 3 weeks previously	None
Patient	Slight prostration. Fever 99-100	Throat deeply injected, no ulceration	4-6 colonies to each blood-agar plate
Patient	Slight prostration. Fever 99-100	Throat deeply injected, no ulceration	8-10 colonies to each blood-agar plate
Patient	Severe prostration. Fever 103-104	Ulceration of throat, exudate on tonsils	Large numbers to each plate
A patient who did not use Pond's milk	Mild prostration, slight fever	Throat injected, no ulceration	None
Patient	Moderate prostration. Fever 101-102	Throat deeply injected, no ulceration	Almost pure growth
Patient	Marked prostration. Fever 102-104	Throat ulcerated, exudate and pseudomembrane on tonsils	Almost pure growth
Pond's milk (sample received from board of health)			Large numbers

the beta hemolytic type were isolated from the plate cultures from 4 of the patients, from the dairymen who had positive cultures, and from the milk.

A fresh horse-blood-agar plate was made for each streptococcus, and read at the end of 24 hours' incubation, plates containing more than 50 colonies being disregarded. In each instance there was a clear-cut zone of hemolysis, 3 mm. in diameter, at the end of 24 hours. Under low power all the outlines of the red cells in the hemolyzed zone had disappeared.

The colonies in the depths of the medium were disc-shaped, the borders

regular and symmetrical. On meat-infusion-agar slants, the colonies were moist, grayish-white, and discrete. In meat-infusion broth the length of chain was from 8 to 10 cocci, tho from 20 to 30 were sometimes seen in one chain. When freshly isolated, the cocci had a capsular substance, variable in amount, but usually about twice the diameter of the coccus.

TABLE 2

REACTIONS OF THE STREPTOCOCCI FROM THE DORCHESTER EPIDEMIC IN STANDARD SUGAR MEDIA

No. and Source	Type of Hemolysis	Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
3D O. dairy	Typical beta	2.6	2.1	2.7	2.3	1.3	2.7	1.4	1.3
4D F. dairy		2.8	2.3	2.9	2.4	1.2	2.8	1.3	1.3
5D F. dairy		2.1	2.5	2.5	2.9	1.4	3.2	1.4	1.1
11D B. dairy		2.3	2.2	2.7	2.3	1.3	2.4	1.2	1.2
14D Patient		3.0	2.3	3.0	2.1	1.2	2.5	1.3	1.3
15D Patient		3.4	2.2	2.6	2.2	1.3	2.6	1.2	1.3
17D Patient		3.6	2.2	2.5	2.8	1.3	2.3	1.3	1.2
18D Patient		2.8	2.6	2.4	2.4	1.2	2.6	1.3	1.4
19D P. dairy		3.6	2.9	3.2	2.7	1.5	2.8	1.4	1.4

A glance at Table 2 will show that the fermentative reactions of the streptococci from the throats of 4 dairymen, 4 patients, and from the milk were practically identical. The degree of acidity attained in the different carbohydrate media through fermentation was seldom above 3 (3 c.c. normal NaOH per 100 c.c. of media).

Agglutination tests with the streptococci from the throats of the dairymen and the patients, and from the milk also gave identical results (Table 3).

TABLE 3

RESULTS OF AGGLUTINATION TESTS WITH STREPTOCOCCI OF THE DORCHESTER EPIDEMIC

Source of Strain Employed	Dilutions (Serum of rabbit immunized against Streptococcus 8)									Control
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2500	1:5120	
18D Patient	++++	++++	+++	+++	++	++	+	±	—	—
4D F. dairy	c	c	c	++++	++++	+++	+	±	—	—
Pond's milk	++++	+++	+++	+++	++	++	+	±	—	—
K13 Boarding school	+++	++	++	+	+	±	—	—	—	—
S & B (Smith and Brown)	±	±	+++	+++	+	±	—	—	—	—
48N Normal throat	+++	+++	+++	+++	+	±	—	—	—	—
4NY N. Y. epidemic	±	—	—	—	—	—	—	—	—	—
10T Sporadic cases of tonsillitis (Table 15)	—	—	—	—	—	—	—	—	—	—
11T	—	—	—	—	—	—	—	—	—	—

The first three organisms were from the Dorchester epidemic. 48N was a beta hemolytic streptococcus from a normal throat (Table 10). 4NY was from a milk-borne epidemic of tonsillitis in New York state. All these strains were of the beta hemolytic type and all fermented the same carbohydrates.

When 0.5 c.c. of a 24-hour broth culture of the strain from any of the cases was injected intraperitoneally into a mouse, death ensued in from 24 to 48 hours. The injection of 1 c.c. of a 24-hour broth culture intravenously into a rabbit, caused high fever with marked loss of weight, followed as a rule by severe polyarthritis, continued high fever, and continued loss of weight. The temperature returned to normal in from 2 to 3 months. In some cases there was permanent disability, with fixation of the joint.

In summary: The beta hemolytic streptococcus from the throats of patients ill with septic sore throat was identical with that from the throats of the dairymen and from Pond's milk, which had been used by almost 90% of those who were ill. This streptococcus resembled

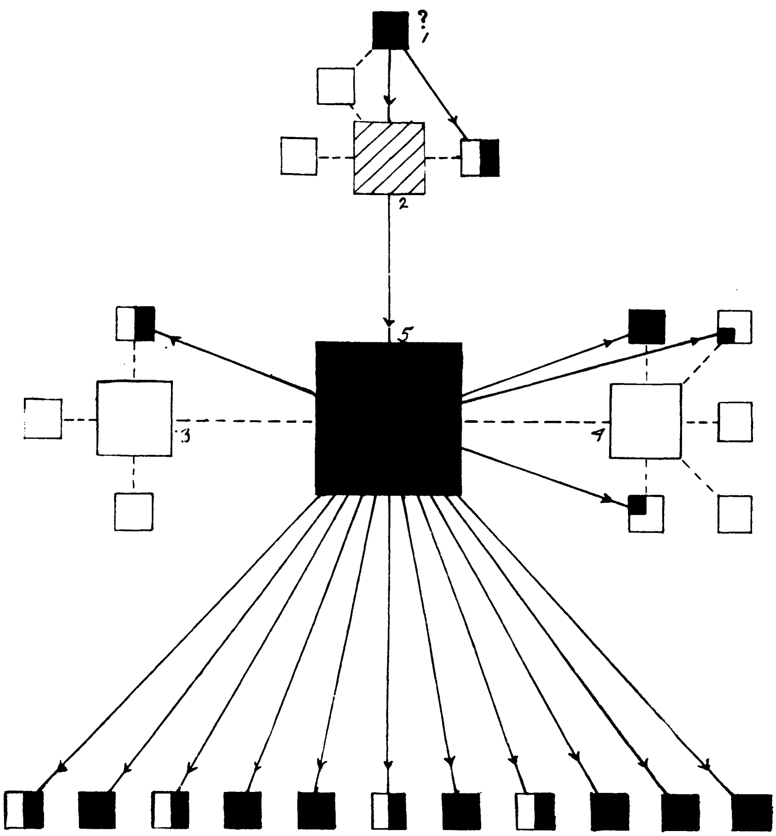

Chart 2. The probable course of the infection in the Dorchester milk-borne epidemic. (1) A. B., a supposed case of scarlet fever. (2) B. farm. (3) C. Farm. (4) F. farm. (5) Pond's distributing station. (6) Cases of septic sore throat. The black squares represent positive cultures of the beta hemolytic streptococcus. The arrows indicate the probable course of the infection.

in all its characteristics the group of streptococci isolated by Smith and Brown in their studies on milk-borne epidemics of septic sore throat.

The puzzling fact of the epidemiologic data was that the beta hemolytic streptococcus should be present in the throats of so many of the dairymen. Thus, one of the milkers of the F dairy, who was ill with septic sore throat, had large numbers of the beta hemolytic

streptococcus in his throat, but two milkers of the same dairy who had normal or but slightly affected throats, also had a few of the beta hemolytic streptococcus in their throats. At least one worker on each farm gave a positive culture of this streptococcus.

The probable explanation is that the workers of the F. farm and of the C. farm had been infected in the same way as the patients; namely, through the milk from the B. farm. The probable course of the infecting organism is shown in Chart 2. A.B., convalescing from scarlet fever (?), infected one of the cows on his father's farm with a beta hemolytic streptococcus. This cow contaminated the milk which was sent to Pond, to be further mixed with the F. and C. milk, and delivered. Thus the bottled milk became contaminated. Bottles returned to C. and F. by Pond caused infection of the milkers on the C. and F. dairies.

There are 3 weak points in these epidemiologic studies as shown in the diagram: (1) A sample of milk was not obtained from each cow of the B. farm, or of the B. milk. (2) A positive culture was not obtained from A.B., the supposed source of infection. (3) We have no proof that one of the other farms did not contaminate Pond's milk. We feel sure that E.F. of the F. farm and T.B. of the C. farm were not the source of the infection, since they developed sore throat at the same time that the other patients did. We have no knowledge as to the date of infection of G.L. or A.L. of the F. dairy. The epidemiologic evidence is therefore not conclusive. We are sure only that the epidemic was due to the beta hemolytic streptococcus, and that the source of infection was Pond's milk.

Additional facts of interest are that the same streptococcus may cause severe, moderate, or mild tonsillitis, and that the beta hemolytic streptococcus may be found in throats that are clinically normal, as in the cases of G.L. and A.L.

AN EPIDEMIC OF TONSILLITIS AT THE K. BOARDING SCHOOL

Of most importance in the study of an epidemic is ability to control, within a small compass, all possible factors pertaining to it. The epidemic of tonsillitis at the K. boarding school is for this reason of special interest. The epidemic was traced to infected milk that had been insufficiently pasteurized. The organism isolated from the throats of the patients was isolated also from the udder of one of the farm cows. It was identical with the one described by Smith and Brown as

a human strain of streptococcus. The only missing factor in the epidemiology of the outbreak was the individual who had infected the cow.

EPIDEMIOLOGIC STUDIES

There were about 160 boys in this school, living under ideal conditions for the preservation of health. There had been no sickness in the school, with the exception of one boy who had had tonsillitis 14 days before. He had been isolated at once, kept in the hospital, with his own dishes, food, attendants, etc. This case is shown in the incidence chart and included in the studies because he was considered by some to be the source of the infection.

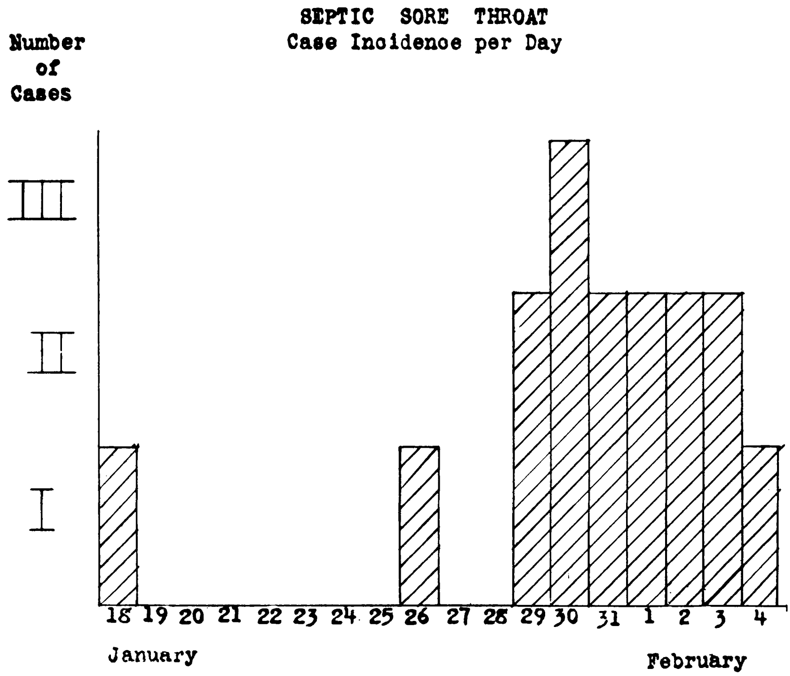

Chart 3. Case incidence of the epidemic at the K. boarding school.

There were 17 cases in the epidemic (Chart 3). A summary of each case is given in Table 4 with the clinical history and the preliminary laboratory findings.

Beta hemolytic streptococci were isolated from the throats of 15 of the boys. Four of these boys had severe septic sore throat with high fever, marked prostration, ulceration of the tonsils, pseudomembrane on the tonsils, and angina of the throat. Their convalescence was slow. Six were moderately ill with what would have been classed as ordinary tonsillitis. Four had slight sore throat with no ulceration of the tonsils and little or no fever. One had slight sore throat, but later developed severe otitis media.

All factors that could play a part in the spread of the outbreak were con-

TABLE 4

SUMMARY OF THE CLINICAL DATA, AND PRELIMINARY LABORATORY FINDINGS IN THE EPIDEMIC AT THE K. BOARDING SCHOOL

Patient	General Condition	Local Condition	Day of Disease	Total Colonies per Plate	Hemolytic Colonies	Type of Hemolysis	Diameter of Hemolytic Zone, mm.
K1	Temp. 100-102 for 5-6 days. Moderate prostration	Ulceration of the tonsils. Moderate edema of the neck	13	150–200	75–100	Alpha type	1
K2	Temp. 101-102. Marked prostration	Marked ulceration with exudate	7	200–250	Almost pure growth	Beta	3
K3	Temp. 104. Severe prostration	Severe sore throat. Ulceration on tonsils with membrane. Moderate edema of neck	2	150–200	Almost pure growth	Beta	3
K4	Temp. 100. Moderate prostration	Throat moderately sore. No edema of the neck. No ulceration of tonsils	1	200–250	Almost pure growth	Beta	3
K5	Temp. 99.5. Slight prostration. Recovery in 2-3 days	Slight sore throat. No ulceration of the tonsils	1	150–175	20–30	No beta	1.5–2
K6	Temp. 100. Moderate prostration	Tonsillar crypts filled with exudate. No ulceration. No edema of the neck	4	100–125	Almost pure growth	Beta	3
K7	No fever. No prostration. Slight nasopharyngitis for past 10 days	Throat somewhat sore for past 3-4 days. Throat moderately injected	4?	250–300	Large numbers	All alpha type. No beta	1-2
K8	Temp. 101.5. Moderate prostration	Ulceration and slight exudate on tonsils. Deep injection of whole throat	2	150–200	Almost pure growth	Beta	3
K9	Temp. 101. Moderate prostration	Moderately sore throat. No ulceration of tonsils. No edema of the neck	6	75–100	50–60	Beta	3

sidered. The probability of contact infection seemed unlikely, because each boy was isolated as soon as he developed sore throat or fever. The only possible contact cases were K1 and K2. K1 was not considered a likely cause of the outbreak, because he had contracted the disease during the Christmas holidays, was ill on the day of his return to school, had been completely isolated from the first, had recovered from his tonsillitis, but had developed myocarditis, and was in his third week of isolation when the epidemic began. Furthermore, cultural studies of his throat did not show the beta type of hemolytic streptococcus.

The epidemic began with Case K2. The boy in this case was isolated at once. That he had got his infection from the same source as the other boys seemed probable, inasmuch as he had not been away from the school, nor exposed to sources of infection other than those to which the whole school had been exposed.

The milk, which was delivered in bulk from a farm nearby, had been

TABLE 4

SUMMARY OF THE CLINICAL DATA, AND PRELIMINARY LABORATORY FINDINGS IN THE EPIDEMIC AT THE K. BOARDING SCHOOL—*Continued*

Patient	General Condition	Local Condition	Day of Disease	Total Colonies per Plate	Hemolytic Colonies	Type of Hemolysis	Diameter of Hemolytic Zone, mm.
K10	Temp. 99.4. No prostration. Recovery in 4-5 days	Slightly sore throat. No ulceration of tonsils	5	75–80	10–15	Beta	3
K11	Temp. 99. No prostration	Throat slightly sore. Deep injection but no ulceration. Normal in 3 days	6	150–200	Almost pure growth	Beta	3
K12	No fever or prostration until complications	Slightly sore throat. Used a nasal douche, and 2 days later developed severe otitis media	5	Throat 150–200 Ear 200–250	Throat 10–15 Ear, almost pure growth	Throat, beta Ear, beta	3 3
K13	Temp. 102.4. Moderate prostration. Well in 3-4 days	Throat moderately sore. Moderate injection with exudate. No ulceration	5	100–150	50–60	Beta	3
K14	Temp. 100.4. Moderate prostration. Recovery in 6-7 days	Throat moderately sore. Deep injection, but no ulceration	3	200–250	Almost pure growth	Beta	3
K15	No fever. No prostration	Throat slightly sore, with slight injection, but no exudate and no ulceration	4	150–200	Almost pure growth	Beta	3
K16	Temp. 99.3. No prostration	Throat slightly sore, with slight injection, but no exudate and no ulceration	3	150–200	10–15	Various types No beta	
K17	No fever. No prostration	Throat slightly sore. Moderate injection. No ulceration	2	75–100	7–8	Beta	3
K18	Temp. 99.8. No marked degree of prostration	Throat deeply injected. No exudate, or ulceration	4	100–125	4–5	Beta	3

inadequately pasteurized in the school kitchen. Five-gallon cans of cold milk were placed in a vat of water, and the water heated to 170 F. for 20 minutes; the contents of the cans at the end of 20 minutes, as shown by subsequent tests, had been raised only to incubation temperature. The dairy was in an unsanitary condition, and 4 of the 50 cows in the dairy were found to have mastitis:

Cow 1.—General condition excellent. Left hind quarter of the udder slightly tender, with slight inflammation, no pus found in the milk. One small platinum loop (2 mm.) of milk from this udder added to 10 c.c. of blood agar and plated at the end of 48 hours showed in each plate from 8 to 10 colonies of hemolytic streptococci of the beta type (Table 5).

Cow 2.—Had been drying up for 2 or 3 weeks. Milk not used for several days. Udder inflamed, and milk contained large amounts of pus. No hemolytic streptococci on blood-agar plates.

Cow 3.—"Garget" 1 week, during which milk had not been used. Left hind quarter of the udder inflamed, but no pus in the milk. No streptococci of the beta type in cultures from this sample.

Cow 4.—Condemned as tuberculous 1 week before, but milk was still being used. Definite tuberculosis of left hind quarter of the udder. One small loopful of the milk added to 10 c.c. of blood agar, at the end of 24-48 hours, showed on each plate from 7 to 8 colonies of hemolytic streptococci, not, however, of the beta type. The diameter of the hemolyzed zones was from 1.5 to 2 mm., but hemolysis was not complete. Reports of this study are given in Table 5.

TABLE 5

LABORATORY STUDIES OF THE HEMOLYTIC STREPTOCOCCI OF THE K. BOARDING SCHOOL EPIDEMIC

No.	Type of Hemolysis	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
K8	Beta	0.5 c.c. killed mouse in 36 hours	30-40	2.9	2.8	2.4	2.5	1.3	2.6	1.0	1.0
K7	Not a beta type. Zones 1-2 mm.	0.5 c.c. did not kill mouse	15-20, sometimes longer	2.6	3.7	3.8	3.3	1.4	1.3	1.2	1.4
Cow 1	Beta	0.5 c.c. killed mouse in 30 hours	20-30, sometimes longer	2.8	2.9	2.8	2.7	1.4	2.9	1.3	1.4
Cow 4	Not beta type. Zone 2-2.5 mm.	0.5 c.c. killed mouse in 24 hours	4-8-12	5.9	4.6	4.5	5.1	1.3	1.3	1.4	1.3
K10	Beta	0.5 c.c. killed mouse in 48 hours	30-40	3.3	3.8	3.0	2.7	1.2	2.7	1.3	1.2
K12, ear culture	Beta	0.5 c.c. killed mouse in 36 hours	20-30, some long and interlacing	2.9	2.3	2.7	3.3	1.0	2.8	1.1	1.0
K13	Beta	0.5 c.c. did not kill mouse	Long and interlacing	3.0	3.1	3.0	2.6	1.0	3.3	0.9	1.1
K14	Beta	0.5 c.c. killed mouse in 36 hours	40-50 or more	2.4	2.1	2.8	2.6	1.0	3.0	1.0	1.3
K3	Beta	0.5 c.c. killed mouse in 20 hours	30-40 or longer	3.0	2.9	2.9	2.8	1.0	3.1	1.3	1.2
K3a, 2nd isolation	Beta	0.0 c.c. killed mouse in 48 hours	30-40	2.9	2.7	3.0	2.8	1.2	2.7	1.3	1.2

A summary of the epidemiologic factors compelled us to conclude that the source of the epidemic was the milk, because: (1) Boys of all ages, from all the dormitories, and all the class rooms, were infected. (2) Food was the only factor common to them all. (3) The incidence curve suggested a milk epidemic. (4) The milk had not been pasteurized. (5) Hemolytic streptococci were found in the udders of 2 of the cows on the farm supplying the milk.

BACTERIOLOGIC STUDIES

Studies were now undertaken to identify the streptococci found in the throats of the boys, and to show any relation that might exist between these streptococci and those found in the udders of the diseased cows.

A number of colonies of typical beta hemolytic streptococci were isolated in pure culture from severe, moderate, and mild types of the disease; from the aural discharge of the case with mild sore throat and severe otitis media; and from the milk of the diseased cows. The characteristics noted were: type of hemolysis, appearance on meat infusion agar, length of chain in a 24-hour meat-infusion-broth culture, reactions on the standard carbohydrate media, and virulence for animals. The summary of these results in Table 5 indicates that the microorganism from the throats of the patients and that from the udder of Cow 1 are of the same strain.

The colonies on meat infusion agar in each instance were grayish-white, moist, and raised, growing readily on all ordinary infusion agar without the addition of ascitic fluid. On blood-agar plates there was a clear-cut zone of hemolysis from 2.5 to 3 mm. in diameter at the end of 24 hours. The length of chain in meat infusion broth was from 20 to 30 cocci, often shorter than 20, but seldom longer. There were a few bizarre forms in meat infusion broth (24-hour growth). Capsular substance about twice the diameter of the coccus was seen in freshly isolated strains.

Dextrose, lactose, maltose, saccharose, and salicin were fermented. Mannite, raffinose, and inulin were not. The degree of acidity in the sugar tubes at the end of 7 days was seldom carried beyond 3.

Agglutination tests furnished further evidence that the hemolytic streptococci isolated from the throats of the boys were of the same strain as those isolated from the udder of Cow 1 (Table 6).

The strains isolated from the throats of the boys and from the cow had about the same degree of pathogenicity for animals. Mice were killed in from 36 to 48 hours with the injection of 0.5 c.c. of a 24-hour broth culture into the peritoneal cavity. Rabbits injected with 1 c.c. of a 24-hour broth culture intravenously developed high fever, and severe polyarthritis, the arthritis lasting for several weeks and resulting in some instances in permanent disability.

The last link in the chain would have been the discovery of the source of infection of the cow. Cultures from the throats of the men who were connected with the production of the milk gave a hemolytic streptococcus in only one instance, Culture K7, Table 4. This man had had a slightly sore throat, but he had not been prostrated or feverish. This hemolytic streptococcus proved not to be of the same strain as those isolated from the throats of the boys or from the udder of the cow (Table 5). We are therefore unable to show the probable source of infection of the cow.

Throat cultures from 5 boys after their temperature had returned to normal and they were up and about the hospital ward, or returned to their dormitory, yielded hemolytic streptococci of the same cultural

TABLE 6

RESULTS OF AGGLUTINATION TESTS WITH STREPTOCOCCI FROM THE MILK-BORNE EPIDEMIC OF SEPTIC SORE THROAT AT THE K. BOARDING SCHOOL

Source of Strain	Dilution of Serum									
	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5720	Control
	Using rabbit serum immunized against the streptococcus from Cow 1									
Cow 1	+++	+++	+++	+++	++	+	+	±	−	−
Human (4)	+++	+++	+++	++	++	++	++	+	−	−
Human (7)	+++	+++	+++	+++	++	++	±	−	−	−
Human (13)	+++	+++	+++	++	+	±	±	±	−	−
	Using rabbit serum immunized against the streptococcus from Cow 4									
Cow 4	+++	+++	+++	++	++	++	++	+	±	−
Cow 1	++	+	±	−	−	−	−	−	−	−
Human (7)	++	+	±	±	−	−	−	−	−	−
Human (4)	+	+	±	−	−	−	−	−	−	−
Human (13)	++	+	+	−	−	−	−	−	−	−
	Using rabbit serum immunized against the streptococcus from Human Strain 4									
Cow 1	+++	+++	+++	++	+	+	+	+	±	−
Human (4)	+++	+++	+++	++	++	++	++	++	±	−
Human (7)	++	++	+	++	++	+	±	±	−	−
Human (13)	++	++	++	++	+	+	±	±	−	−
Blood from a case of fatal scarlet fever	±	−	−	−	−	−	−	−	−	−

TABLE 7

PERSISTENCE OF THE BETA HEMOLYTIC STREPTOCOCCUS IN THE THROATS OF PATIENTS CONVALESCENT FROM SEPTIC SORE THROAT

Patient	Present General Condition	Present Local Condition	Degree of Severity of Disease	Day of Disease	Total Number of Colonies	Number of Hemolytic Colonies	Type of Hemolysis	Diameter of Zone, mm.
K2	Temp. normal. Returned to dormitory	Throat normal	Severe	12th	200–250	3–4	Beta	3
K3	Still in bed. Moderate prostration. No fever	Glands of neck large and tender. Throat still slightly injected	Severe	8th	150–200	Almost pure growth	Beta	3
K4	Well. Discharged to dormitory 2 days ago	Slight injection of throat. Glands of neck still tender	Moderate	7th	150–175	40–45	Beta	3
K6	Well. Discharged to dormitory 3 days ago	No injection of throat	Moderate	10th	200–250	10–12	Beta	3
K8	Headache and prostration persist. No fever	Deep injection of throat. Tonsils large and ragged	Moderate	8th	200–250	Almost pure growth	Beta	3

characteristics and of the same virulence for animals as the original strains (Table 7). Inasmuch as these streptococci were present in the throats 10 days after acute symptoms had subsided, it seems probable that, in some instances at least, the virulent beta hemolytic streptococcus may remain in the throat of an individual after recovery from the disease, for weeks or even months.

These facts suggest also that the disease is not readily spread by contact, else more boys would have developed the disease from contact with the boys who returned to the dormitories carrying the infecting organism in their throats.

In summary: The same strain of the beta hemolytic streptococcus was isolated from the severe, moderate, and mild cases of septic sore throat at the K. boarding school. That is, "septic sore throat" may occur as a mild transient disease.

This strain was identical with the strain of beta hemolytic streptococci isolated from the udder of Cow 1 on the farm supplying the school with milk.

These strains of streptococci belong to the same group of streptococci that Smith and Brown isolated in their studies of epidemics of septic sore throat.

THE PRESENCE OF THE BETA HEMOLYTIC STREPTOCOCCUS IN NORMAL THROATS

Studies of the cultures of the epidemic of septic sore throat at Dorchester and the K. boarding school indicated that the beta hemolytic streptococcus may sometimes be found in throats that are clinically normal. This fact suggested a study of the occurrence of beta hemolytic streptococci in the throats of normal individuals, and in the throats of individuals who had had septic sore throat within 2 years; that is, as carriers.

METHOD OF STUDY

Cultures were taken from the throats of 100 normal individuals in the population at large, and studied on blood-agar plates by a technic similar to that used in the previous studies. Swabs were taken of the tonsils and nasopharynx and placed at once in 10 c.c. of normal salt solution. One or two large platinum loopfuls (4 mm.) of the suspension, instead of one small loopful as in the previous studies, were transferred to blood agar, the blood-agar plates being made with 10 c.c. of meat infusion agar, + .8 to + 1, with 1 c.c. of defibrinated horse blood added. The blood-agar plates were incubated at 37.5 C. and read at the end of 24 and 48 hours.

Colonies on these plates which were suggestive of the beta type were isolated in broth, and a pure culture studied on the blood-agar plate. If the strepto-

coccus cultures thus obtained in pure growth resembled in any way the beta type of hemolytic streptococcus, they were studied further as to carbohydrate reactions, virulence for animals, and other characteristics (see Tables 9 and 10).

One hundred average throats, rather than 100 normal throats, really formed the basis of this study; many of the individuals had enlarged tonsils, slight injection of the nasopharynx, or a past history of some throat trouble.

The clinical data considered were: age, tendency to sore throat, tendency to attacks of tonsillitis, date of last attack of tonsillitis, history of scarlet fever, history of septic sore throat, and present appearance of the throat. The preliminary laboratory data recorded were: the estimation of the total number of colonies on the blood-agar plate, the estimation of the total number of hemolytic colonies on each blood-agar plate, and the estimation of the number of colonies on each blood-agar plate which were suggestive of the beta type of hemolysis. A summary of the findings is presented in Table 8.

TABLE 8

HEMOLYTIC STREPTOCOCCI SUGGESTIVE OF THE BETA TYPE IN NORMAL THROATS

Summary of 100 Cases	Adults	Children Under 13 Years
Total number of cases	66	34
Number subject to sore throat	15	10
Number subject to tonsillitis, one attack every year or oftener	11	10
Number having had nasopharyngitis within 1 or 2 months	15	5
Number having had tonsillitis within 2 years	20	13
Number having had scarlet fever	9	2
Number having had septic sore throat	1 (2 yr. ago.)	1 (6 mo. ago)
Number showing hemolytic streptococci present	32	18
Number of those who had had tonsillitis within 2 years, who showed hemolytic streptococci present in throats	7	5
Number of those not subject to tonsillitis who showed hemolytic streptococci present in throats	13	11
Number of those who had had nasopharyngitis (colds) within 2 months, who showed hemolytic streptococci present in throats	8	2

An analysis of the cultural studies (Tables 9 and 10) shows only 1 streptococcus, No. 48, to be of the beta hemolytic type with all the typical characteristics of the Smith classification; that is, beta type of hemolysis, characteristic carbohydrate reactions, and virulence for animals. This organism came from a young man who had recently returned from an ocean cruise, and was in a hospital with a severe subacute nephritis. There was no history of scarlet fever nor of tonsillitis, and his throat appeared normal. There were two or three typical beta hemolytic colonies on each primary blood-agar plate.

Cultures from Cases 31 and 35 are interesting, having typical hemolysis and characteristic carbohydrate reactions, but no virulence for animals. Streptococci from Cases 80, 89, and 96 did not ferment lactose, being similar in this respect to the type isolated by Smith and Brown in their Outbreak A.

In summary: Hemolytic streptococci were found in about 50% of normal throats in sufficient numbers to be detected on blood-agar plates made from throat swabs.

Beta hemolytic streptococci of the Smith type were found in 1 of 100 normal throats studied.

TABLE 9

RESULTS OF STUDIES OF AVERAGE THROATS THAT HARBORED STREPTOCOCCI SUGGESTIVE OF BETA HEMOLYSIS

No.	Age	Clinical History	Appearance of Throat	History of Scarlet Fever	Hemolysis		
					Preliminary Plate		Isolation Plate
					Total Colonies	Colonies Suggestive of Beta Hemolysis	
N31	21	Tonsillitis once a year. Last attack 1½ years before	Tonsils large and ragged	No	200	6	Pure culture, typical beta type; zone 3 mm. wide
N35	29	Working in laboratory with the beta type. Self-infected 3 months before	Apparently normal	No	100–125	10–11	Typical beta type; zone 2.5 mm. wide
N48	18	Ill with subacute nephritis. Never had had tonsillitis	Apparently normal	No	150–200	2–3	Typical beta type; zone 3.5 mm. wide
N59	45	Severe tonsillitis 14 years before. Slight sore throat 1 week before	Slight pharyngitis	No	100–150	1–2	Suggestive of beta type; zone 3.5 to 4 mm. wide; colonies very small
N80	10	Severe tonsillitis 6 months before. Not well since. Came to hospital for tonsillectomy	Tonsils large and ragged. Throat injected	No	200–250	11	Not typical of beta type; incomplete hemolysis just at colony border
N83	14	Subject to tonsillitis. Had septic sore throat with 4 others of her family 8 months before	Apparently normal	No	150–175	4–5	Zone is clear-cut but narrow; 2 mm. wide
N89	12	Not subject to tonsillitis	Apparently normal	No	75–80	4–5	Suggestive of beta type; incomplete hemolysis near colony
N95	8	Subject to tonsillitis. Last attack 2 years before	Tonsils not remarkable	No	100–150	1–2	Zone 3.5-4 mm. wide; slightly granular zone near colony
N96	3	Not subject to tonsillitis	Apparently normal	No	150–175	3–4	Incomplete hemolysis near colony
N97	10	Subject to tonsillitis. Last attack 6 weeks before. Tonsils removed 4 weeks before	Throat injected	No	150–175	4–5	Zone 2 mm. wide; not typical; incomplete hemolysis

These studies have shown no direct relation between the length of chain of a streptococcus and its virulence for animals. This fact is mentioned because length of chain in a milk streptococcus is used by some municipal and state laboratories as an index to pathogenicity.

TABLE 10

CULTURAL CHARACTERISTICS OF STREPTOCOCCI SUGGESTIVE OF BETA TYPE ISOLATED FROM 100 AVERAGE THROATS

No.	Beta Type of Hemolysis	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Sugar Reactions							
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
N31	Typical	0.5 c.c. did not kill mouse	8-12-16, sometimes longer	4.0	3.6	3.5	3.5	1.2	3.8	1.2	1.2
N35	Typical	0.5 c.c. did not kill mouse	8-12, never more	4.8	4.0	4.3	4.0	1.3	3.8	1.4	1.3
N48*	Typical	0.5 c.c. killed mouse in 24 hours	Interlacing masses of chains	3.5	2.3	3.8	2.8	1.1	2.3	1.2	1.3
N59	Suggestive	0.5 c.c. did not kill mouse	8-12-16, some 30-40	3.4	3.5	3.2	3.6	0.9	0.9	1.0	1.0
N80	Suggestive	0.5 c.c. did not kill mouse	4-8-12, seldom longer	5.4	1.0	4.8	5.4	1.1	4.5	1.1	5.2
N83	Not typical; zone clear-cut, but narrow	0.5 c.c. did not kill mouse	Long interlacing masses of chains	2.8	2.7	3.0	3.3	1.4	2.5	1.3	1.3
N89	Suggestive but not typical	0.5 c.c. killed mouse in 48 hours	4-8-12, some 20-30	2.4	1.3	2.1	2.4	1.2	3.0	1.2	1.2
N95	Suggestive but not typical	0.5 c.c. killed mouse in 7 days	4-8-12, some 20-30	3.0	2.5	2.8	2.5	1.0	3.4	1.3	1.4
N96	Suggestive but not typical	0.5 c.c. did not kill mouse	8-12, some longer	3.0	1.5	2.8	2.7	1.4	2.5	1.3	1.4
N97	Suggestive but not typical	0.5 c.c. killed mouse in 8 days (pericarditis)	4-8-12, many bizarre forms	5 6	5.8	4.4	5.6	1.4	4.1	4.7	4.5

* 0.5 c.c. of a 24-hour broth culture injected intravenously into a rabbit caused high fever and death in 6 days.

AN EPIDEMIC DUE TO INFECTION BY CONTACT WITH CARRIERS OF THE BETA HEMOLYTIC STREPTOCOCCUS

The study of the epidemic at the K. boarding school suggested that the beta hemolytic streptococcus might remain in the throat of an individual long after recovery from the disease, and the history of patients

who have had septic sore throat tends to substantiate this belief, since in many instances there is chronic inflammation of the throat. The danger, if any, of such persons to the community, tho it has been assumed, has not been studied.

EPIDEMIOLOGIC STUDIES AT THE B. HOSPITAL

In January, 1915, there was an epidemic of septic sore throat at the B. hospital, many of the hospital employees being ill. Some of the cases were of the severe type, complicated in 2 cases by joint-involvement; some were

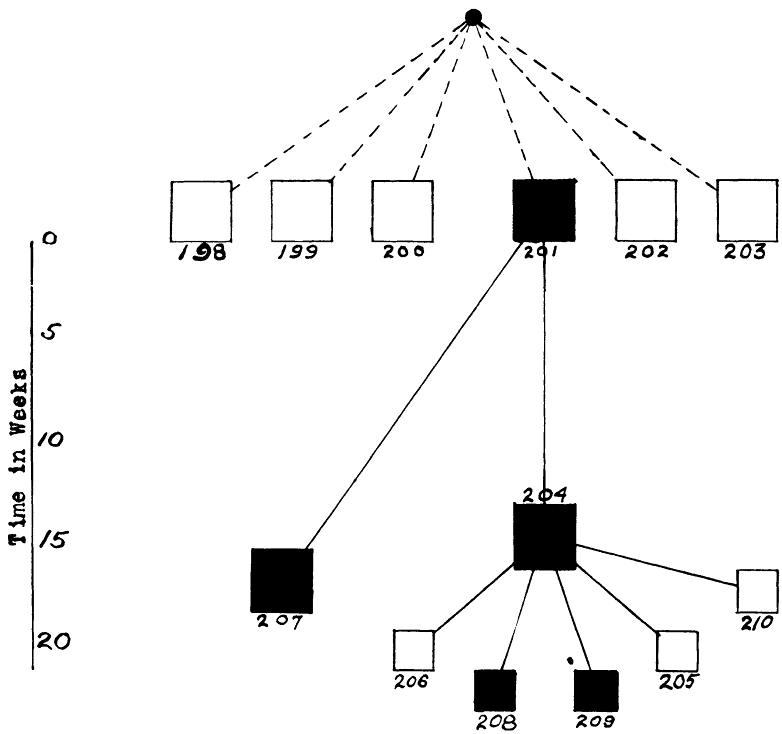

Chart 4. The probable course of a contact epidemic of septic sore throat at the B. hospital. The squares represent cultures taken. Black squares indicate positive cultures of beta hemolytic streptococci. Large squares denote employees of the hospital.

The squares numbered 198 to 203 represent cases of the milk-borne epidemic in January. Case 201 became a carrier and infected 204 and also probably 207. Case 204 probably infected the patients, since she as night nurse came in contact with all of them and had more to do with the preparation of their food than had the day nurses.

of moderate severity, and some were mild; in all the recovery was slow. The hospital authorities assumed the epidemic to be milk-borne and changed the supply of raw milk which had been used to pasteurized milk. No further cases developed.

In April, 1915, I was asked to investigate conditions at the hospital. Of every 10 patients admitted to the hospital 1 or 2 would develop tonsillitis on the 3rd or 4th day after admission. Some of the nurses also had the disease,

TABLE 11

CLINICAL CONDITION AND PRELIMINARY LABORATORY FINDINGS IN CONNECTION WITH POSITIVE CULTURES FROM THE B. HOSPITAL EPIDEMIC

No.	Source	Date of Disease	Clinical Condition: General	Clinical Condition: Local	History of Tonsillitis	Type of Hemolysis	Hemolytic Colonies to Each Plate
201*	Day nurse	3 months before	Had had a severe infection. Now in excellent health	Throat somewhat sore at times since the epidemic. Tonsils slightly injected	Never subject to sore throat until epidemic	Typical beta	10–15
204	Night nurse	3 weeks before	Felt tired and run down since attack. Had had a septic sore throat	Throat injected. Tonsils large and ragged	Never subject to tonsillitis until this attack	Typical beta	25–30
207	Day nurse	1 week before	Had had moderate attack. Temp. 101-102. Had not yet regained strength	Throat injected. Tonsils ragged and inflamed. Slight glandular enlargement at angle of jaw	Had had tonsillitis once or twice within the past 5 years	Typical beta	50–60, almost pure culture
208	Patient	3 days before	Severe attack. Marked prostration. Temp. 102-103	Throat ulcerated. Moderate enlargement of glands of neck	Never had had tonsillitis before	Typical beta	Large numbers, almost pure culture
209	Patient	3 days before	Moderate prostration. Temp. 100-101	Throat injected and very sore. No ulceration. Moderate glandular enlargement	Subject to tonsillitis. Last attack 6 months before	Typical beta	Large numbers, almost pure culture

* 1 c.c. of a 24-hour broth culture injected intravenously into a rabbit caused high

but in no instance did a second attack occur in an individual who had had the disease in January.

The food of the patients and nurses was found to be satisfactory. The milk and cream were efficiently pasteurized. Furthermore, the incidence of the cases suggested contact infection, with a carrier as the causal agent.

Throat cultures were taken from all those who came in contact with the patients, including the cook, waitresses, nurses, attendants, etc. Blood-agar plates were made from the throat swabs by the same technic as heretofore described (Table 11). It was evident that the beta hemolytic streptococcus had caused the infection (see Chart 4). It was found in large numbers in the throats of patients who were acutely ill, and in less numbers in the throat of a nurse who had had the infection recently, and in the throat of a nurse who had been a part of the epidemic 3 months previously.

Cases 198 to 203 (Chart 4) represent cultures from the employees who had had septic sore throat in January. At the time of study only one of these had the beta type of streptococcus in her throat—Case 201. Ever since the epidemic she had had trouble with the throat—tenderness and pain on swallowing, particularly in the morning. There was a slight enlargement of the nodes at the angle of the jaw. This nurse did not come into contact with all the patients, and but one of her patients had tonsillitis. She did come in contact with Nurses 204 and 207, and probably had infected them, one 3 weeks,

TABLE 11—*Continued*

CLINICAL CONDITION AND PRELIMINARY LABORATORY FINDINGS IN CONNECTION WITH POSITIVE CULTURES FROM THE B. HOSPITAL EPIDEMIC

Diameter of Colonies, mm.	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Sugar Reactions							
			Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
2.5	0.5 c.c. killed mouse in 10 days (sticky peritonitis)	Long interlacing chains. 50–100 or more	2.3	2.0	2.0	2.1	1.1	2.0	1.2	1.2
2.5	0.5 c.c. killed mouse in 6 days	Long interlacing chains	2.5	2.4	2.6	2.3	1.2	2.3	1.1	1.0
2.5	0.5 c.c. did not kill mouse	Long interlacing chains, none shorter than 40-50	2.6	2.6	2.4	2.0	1.3	2.0	1.3	1.0
2.5	0.5 c.c. killed mouse in 7 days (sticky peritonitis)	Long interlacing chains. Few short chains. 8-12	2.4	2.0	2.3	2.2	1.0	2.5	1.2	1.3
2.5	0.5 c.c. did not kill mouse	Long interlacing chains. A few short chains	2.5	2.5	2.6	2.4	1.3	2.0	1.2	1.3

fever, followed in 5 days by polyarthritis.

and one 1 week previously. The patients in the ward did not begin to have tonsillitis until after Nurse 204 had recovered from tonsillitis and was back on duty. As night nurse she came into contact with all the patients, and probably was the source of the subsequent infection in patients 205, 206, 208, and 209. The latter two, who were acutely ill at time of visit, showed the presence of the beta hemolytic streptococcus in their throats in large numbers.

In summary: The beta hemolytic streptococcus may be found in the throat of an individual 3 months or more after an attack of septic sore throat. Such a carrier may be a source of infection for other persons, particularly if he comes into close personal contact with them.

CARRIERS. A STUDY OF THROAT CULTURES FROM 20 INDIVIDUALS WHO HAD HAD SEPTIC SORE THROAT WITHIN 2 YEARS PAST

The contact epidemic at the B. hospital strongly suggested that certain individuals harbor the beta hemolytic streptococcus in their throats for a long time after acute symptoms have subsided. This fact was also suggested by the continued presence of this streptococcus in

TABLE 12

CLINICAL AND PRELIMINARY LABORATORY DATA IN THE CASE OF PERSONS WHO HAD HAD SEPTIC SORE THROAT WITHIN 2 YEARS PAST

No.	Age	Time Elapsed Since Attack (mo.)	Degree of Infection	Present Condition	Hemolysis on Preliminary Plate			
					Macroscopic Appearance	Microscopic	Hemolytic Colonies per Plate	Diameter of Zone (mm.)
201B	25	3	Severe	Throat troublesome ever since attack	Beta type	Clear zone	10–15	2.5
99B	24	3	Severe Temp. 104. In bed 3 weeks	Throat normal apparently	Not beta type		40–50	1
17K	13	10	Moderate	Well since	Alpha type	Incomplete	20–30	
16K	12	10	Slight	Well since	Not beta type	Incomplete	10–12	
15K	18	10	Slight	Well since	Not beta type	Incomplete	4–5	
13K	14	10	Moderate	Well since			0	
12K	12	10	Slight	Well since			0	
11K	17	10	Moderate	Well since	Not beta type	Incomplete	8–9	3–4
7K	14	10	Moderate	Slow convalescence; well all last summer (1916)	Suggestive of beta type	Incomplete	6–7	5–6
3K	10	10	Severe	Well for past 6 months	Not beta type		3–4	
221	30	24	Severe	Well since	Not beta type		5–6	3–4
84N	3	8	Moderate	Well since			0	
83N	14	8	Severe	Well since	Suggestive of beta type	Complete	6–7	1.5–2
15D	7	8	Severe. Slow recovery	Not well since epidemic. 2-3 attacks of tonsillitis. Tonsils large, injected	Not beta type		75–100	0.5–1
21D	13	8	Severe. No complications	Well since	Not beta type	Incomplete	4–5	3–4
22D	10	8	Severe	Well since	Not beta type	Incomplete	8–10	1.5–2
23D	8	8	Severe	Tonsils large but not injected	Not beta type	Incomplete	4–6	1.5–2
24D	4	8	Severe	Well since	Not beta type	Incomplete	5–6	2–3
25D	6	8	Moderate	Tonsils ragged. No injection			0	
26D	14	8	Severe	Well since	Alpha type		15–20	

TABLE 13

RESULTS OF LABORATORY STUDIES OF STREPTOCOCCI SUGGESTIVE OF THE BETA HEMOLYTIC TYPE FROM THE THROAT OF INDIVIDUALS WHO HAD HAD SEPTIC SORE THROAT WITHIN 2 YEARS PAST

No.	Type of Hemolysis	Virulence for Animals (24-hr. broth culture)	Length of Chain	Sugar Reactions							
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
201B	Typical beta	0.5 c.c. killed mouse in 10 days	Long interlacing chains	2.5	2.0	2.0	2.1	1.1	2.0	1.2	1.2
83N	Not typical beta. Hemolysis complete but zone narrow	0.5 c.c. did not kill mouse	Long interlacing masses of chains	2.8	2.7	3.0	3.3	1.4	2.5	1.3	1.3
7K	Not typical beta. Zones 8 mm. Hemolysis incomplete	0.5 c.c. killed mouse in 3 days	Chains short. 8-10 some 20-30, but not longer	3.2	2.8	3.0	3.4	1.2	3.0	1.4	1.3

the throats of the boys of the K. boarding school after their temperatures had been normal for a week or two, and they had returned to their dormitories.

Twenty cultures were taken from the throats of persons who had had, within the past 2 years, unmistakable septic sore throat due to the use of contaminated milk. Any colonies on the original plate that were at all suggestive of the beta type of hemolysis were isolated in pure growth and their characteristics determined as regards reaction on carbohydrate media, length of chain, virulence for animals, etc.

The clinical data and preliminary laboratory findings are given in Table 12. Three cultures only were suggestive of the beta type of hemolysis (Table 13). Of these, only No. 201B was a definite beta hemolytic streptococcus. In this case, which has already been referred to in the discussion of the B. hospital epidemic, 3 months had elapsed at the time of study since the attack, and the patient during this time had suffered from irritation of the throat.

Chronic "irritation" of the throat after epidemic tonsillitis is not uncommon. In the K. boarding school, the attending physician of Case K3 writes: "Following the initial attack, the boy had chronic irritation of the throat, swelling of the glands of the neck, and was in a general run-down condition. Complete tonsillectomy three months after the infection resulted in ultimate recovery."

In summary: The greater proportion of cases of septic sore throat do not harbor the beta hemolytic streptococcus for a long time after recovery.

Certain individuals may retain the hemolytic streptococcus in the throat for 3 or 4 months after an attack of the disease.

TABLE 14

CLINICAL AND PRELIMINARY LABORATORY DATA IN CASES OF SPORADIC TONSILLITIS

No.	Age (yr.)	Day of Disease	Clinical Data		Complications	Type of Hemolysis
			Constitutional Symptoms	Local Condition		
365	12	2nd	Temp. 99.6. Moderate prostration	Throat injected. Slight exudate but no ulceration of tonsils	None	Not typical beta
366	9	2nd	Temp. 99.8. Moderate prostration	Slight ulceration with exudate on tonsils	None	Suggestive of beta
367	30	3rd	Temp. 99-100. Slight prostration	Tonsils deeply injected. No ulceration	None	Alpha
368	5	4th	Temp. 99-100	Slight exudate with ulceration on tonsils	None	Beta
369	21	2nd	Temp. 100. Moderate prostration	Throat brilliantly injected. Moderate angina. Slight ulceration	None	Beta
370	9	6th	Temp. 101-102. Moderate prostration	Deep injection of throat. No ulceration	None	Beta
371	21	2nd	Temp. 99.6. Slight prostration	Tonsils injected but not ulcerated	Is subject to tonsillitis	Not beta
372	10	6th	Temp. 102-103. Severe prostration	Tonsils ulcerated with exudate. Glands of neck are tender	None	Beta
373	12	5th	Temp. 99-100. Moderate prostration	Throat injected. No exudate	Scarlet fever (?). Not possible to make a definite diagnosis	Beta
374	8	3rd	Temp. 101-102. Moderate prostration	Tonsils large and injected. Exudate on left	Septic sore throat 8 mo. before. Not well since	Not beta
375	1½	2nd	Temp. 101. Moderate prostration	Throat deeply injected. Exudate on tonsils. Moderate enlargement of glands of the neck	None	Not beta

THE PREVALENCE OF THE BETA HEMOLYTIC STREPTOCOCCUS IN SPORADIC TONSILLITIS

Many observers have shown that acute follicular tonsillitis is associated with a variety of streptococci. Floyd and Wohlbach[16] have demonstrated considerable variation in the cultural characteristics of such strains. In a few cases of sporadic tonsillitis we have applied to the flora from the throats the criteria set forth by Smith and Brown (Table 14).

Twenty cases were chosen, some severe, some moderately ill, and some only mildly so. Six blood-agar plates showed no hemolysis, or only pinpoint hemolytic colonies. Fourteen plates showed hemolytic colonies in large numbers, the diameter of the hemolytic zone varying from 2 to 6 mm. Many of the colonies were not even suggestive of

[16] Jour. Med. Research, 1914, 29, p. 493.

TABLE 14—*Continued*

CLINICAL AND PRELIMINARY LABORATORY DATA IN CASES OF SPORADIC TONSILLITIS

No.	Age (yr.)	Day of Disease	Clinical Data: Constitutional Symptoms	Clinical Data: Local Condition	Complications	Type of Hemolysis
376	18	3rd	Temp. 103-104. Severe prostration	Tonsils covered with exudate. Marked angina	Very slow convalescence	Beta
377	25	6th	Temp. 100-101. Moderate prostration	Tonsils large and injected. No exudate	Is subject to tonsillitis. Present attack a relapse from a more severe one	Not typical
378	18	6th	Temp. 100-101. Moderate prostration	Tonsils large and deeply injected. Exudate on right tonsil	None.............	Alpha
379	13	2nd	Temp. 102. Moderate prostration	Exudate with ulceration on tonsils. Moderate angina	Is subject to tonsillitis. Present attack a relapse	Beta
380	42	4th	Temp. 103-104. Severe prostration	Typical severe septic throat, with exudate, angina, etc.	None.............	Beta
381	4	8th	Temp. 99.6. Slight prostration	Slight ulceration of tonsils. Moderate enlargement of glands of neck	None.............	Suggestive of beta
382	6	5th	Temp. 101. Slight prostration	Throat deeply injected. Slight enlargement of glands of the neck	None.............	Not beta
383	10	3rd	Temp. 103-104. Prostrated	Tonsils ulcerated with heavy exudate	None.............	Beta
384	17	3rd	Temp. 101. Prostrated "Recovered in 3 days"	Tonsils ulcerated. Glands of neck tender and swollen	None.............	Not typical

the beta type, but they were isolated, and their cultural characteristics studied. A summary of these results is shown in Table 15.

Five of the 14 hemolytic cultures corresponded with the type described by Smith and Brown. The other 9 showed incomplete hemolysis, atypical carbohydrate reactions, or a low virulence for animals. Two of the 5 positive strains were from clinically moderate types of tonsillitis, Cases 370 and 379; 3 were from cases severe in type, Cases 372, 376, and 380. Case 380 was diagnosed septic sore throat by at least two clinicians, altho the case occurred sporadically. The more severe cases clinically were as a rule due to the beta type of hemolytic streptococcus; the more moderate cases usually were not. The streptococci from Cases 372, 380, and 381 were similar to the strain isolated by Smith and Brown from Outbreak A, the unusual characteristic being the absence of lactose-fermentation. The strepto-

TABLE 15

RESULTS OF STUDY OF CULTURES FROM 20 CASES OF SPORADIC TONSILLITIS

No.	Type of Hemolysis	Virulence for Animals (0.5 c.c. 24-hr. broth culture)	Length of Chain (cocci)	Sugar Reactions							
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
366	Not typical beta. Small Zone, 1.5 mm.	Killed mouse in 48 hours	8-12-20, seldom more	3.2	3.6	3.8	2.8	1.2	2.6	1.4	1.2
367	Suggestive of beta. Zone, 3-4 mm.	Did not kill mouse	40–50	3.7	3.4	3.2	3.3	2.3	1.3	2.4	1.3
368	Typical beta	Did not kill mouse	8-12, seldom longer	3.8	3.0	3.6	3.4	1.2	1.3	1.0	1.2
369	Beta type. Zones, 4-5 mm.	Killed mouse in 5 days	Very long and interlacing	4.0	3.4	3.3	3.4	2.8	1.4	4.1	3.1
370	Typical beta	Killed mouse in 3 days	30–50	3.0	3.1	3.7	3.0	1.0	2.7	1.3	1.0
371	Alpha	Did not kill mouse	Long and interlacing	2.8	2.5	2.0	2.2	2.4	0.9	1.1	1.2
372	Typical beta	Killed mouse in 48 hours	20–30, seldom longer	3.0	1.2	3.4	3.0	1.0	2.8	1.0	1.0
373	Typical beta	Did not kill mouse	8-12-20, seldom longer	3.4	3.0	3.6	3.2	1.0	1.2	1.1	1.0
376	Typical beta	Killed mouse in 36 hours	40–50	3.4	3.6	3.3	3.2	3.4	3.2	1.0	1.2
377	Not typical Hemolysis incomplete	Did not kill mouse	40-50 or more	3.0	3.1	2.7	3.4	1.3	3.0	1.3	3.4
379	Typical beta	Killed mouse in 36 hours	4–8–12, some 20-30, none longer	2.5	2.4	2.7	3.0	1.4	2.4	1.3	1.4
380	Typical beta	Killed mouse in 24 hours	4–8–12, seldom longer	2.6	1.3	2.5	2.4	1.2	2.4	1.2	1.1
381	Suggestive of beta	Did not kill mouse	4-8-12, not longer	2.5	1.0	2.4	2.5	1.2	2.4	1.3	1.2
383	Beta. Zones, 4 mm.	Did not kill mouse	2-4-8-12, not longer	4.3	3.7	4.2	3.5	1.2	3.6	1.1	1.2

coccus from Case 376 is similar to one strain described by Smith and Brown, in that it ferments both mannite and salicin.

In summary: Sporadic tonsillitis, severe or moderate, may be associated with the streptococcus of the beta hemolytic type described by Smith and Brown.

THE PREVALENCE OF THE BETA HEMOLYTIC STREPTOCOCCUS IN SCARLET FEVER

There is a close relationship between septic sore throat and scarlet fever. Clinically, in septic sore throat, there is an acute onset, with high fever, brilliant injection of the nasopharynx, ulceration of, and false membrane on the tonsils, and in some instances a rash on the skin. In fact, the clinical picture is so similar to that of scarlet fever that in some epidemics part of the cases have been diagnosed as scarlet fever. The etiologic factor in epidemics of septic sore throat is carried by means of contaminated milk. The etiologic agent of scarlet fever may also be carried by milk.[17] In April, 1915, in New York, and also in Dorchester, Massachusetts, there were epidemics of milk-borne septic sore throat, each traceable, not to a case of septic sore throat, but to a case of scarlet fever.

The following small epidemic brings up some interesting questions:

Three little girls had a tea party on Monday afternoon. The children were from different homes, were not in the same room at school, nor in any way exposed to similar conditions. There was no scarlet fever in the neighborhood. On Thursday afternoon all three developed sore throat. At the end of 2 more days, one child presented a typical picture of scarlet fever, with characteristic rash and all the other symptoms. One child had a mild sore throat, slight injection of the nasopharynx, and a mild and somewhat atypical rash. It was with considerable reluctance that the diagnosis of scarlet fever was made. The third child presented the typical picture of septic sore throat, with prostration, high temperature, ulceration of the throat, etc., but with no sign of a rash on the skin. From the throat cultures of each child the beta hemolytic type of streptococcus was isolated. The cultural characteristics, carbohydrate reactions, animal inoculations, and agglutination reactions of these three strains were exactly alike.

Were the first two children infected with something in addition to the beta hemolytic streptococcus, that produced scarlet fever in them? Is it possible that scarlet fever is due to 2 factors? Or did the third child really have scarlet fever without the rash?

It is certain that septic sore throat is due to a streptococcus. Is scarlet fever, after all, due to a streptococcus? Or, if scarlet fever is not due to a streptococcus, what part does the streptococcus play in the course of the disease? What type of streptococcus is found in scarlet fever? Does this type of streptococcus found in the throat of a scarlet-fever patient bear any relation to the mildness or severity of the disease?

METHODS OF STUDY AND RESULTS

Throat cultures from 48 cases of scarlet fever were examined for the presence of the beta hemolytic streptococcus. I have divided these cases into 5 clinical groups: Group I, 4 fatal cases of scarlet fever; Group II, 12 cases

[17] Clark, Jour. Infect. Dis., 1915, 17, p. 109.

of "septic scarlet fever" (severe, with marked prostration, delirium, temperature of 104 F. or higher, ulceration of the throat, edema of the neck, etc.); Group III, 14 cases of moderately severe scarlet fever (with a maximal temperature of 102-103 F., exudate on the throat, but with slight or no ulceration, moderate degree of prostration, etc.); Group IV, 10 cases of mild scarlet fever (with typical rash but mildly injected throat without exudate, temperature 99-100 F., and no prostration); and Group V, carriers (who, tho recovered from the acute symptoms of scarlet fever, had developed a running nose, or discharging ears, the condition persisting for weeks or months).

A swab was taken of the throat and placed in 10 c.c. of normal salt solution. One 4-mm. loopful of this suspension was placed in a second 10-c.c. tube of salt solution, and one 4-mm. loopful of the latter suspension was placed in a 10-c.c. tube of horse-blood agar and plated at once. In this manner an approximate number of beta hemolytic colonies may be estimated, and compared with the numbers found on the plate at different stages of the disease, and in the different groups of cases.

The data are recorded in Tables 16 to 20.

Of 20 cases in which blood cultures were taken, including all the fatal and all the severe cases, only 2 yielded an organism from the blood—a beta hemolytic streptococcus—and in each instance the disease resulted fatally within 2 days.

The Fatal Cases.—In Table 16 are given the results of the studies of 4 fatal cases of scarlet fever. All the patients died in the 2nd week of the

TABLE 16

BETA HEMOLYTIC STREPTOCOCCI FROM FATAL CASES OF SCARLET FEVER

No.	Hemolytic Colonies	Virulence for Rabbits (24-hr. broth culture)	Length of Chain (cocci)	Carbohydrate Reactions: Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin	Time of Culture
19	Almost pure culture	0.5 c.c. caused high fever, septic joints, death	40-50	2.5	3.1	3.6	3.4	1.2	3.8	1.4	1.5	3 days before death
21	Pure culture	0.5 c.c. caused high fever, loss of weight. Recovery after 3 months	10-20, rarely 30	2.1	2.0	2.0	2.5	1.1	2.0	1.0	1.4	After death, from heart blood
35	Almost pure culture, 200-300	1 c.c. caused high fever, joint-involvement, paralysis and death (necrosis of vertebrae)	20-30, rarely longer	3.2	3.4	3.5	2.8	1.4	3.0	1.3	1.2	3 days before death
35	Pure culture	1 c.c. caused high fever, joint-involvement. Recovery in 3 mo.	20-30, sometimes more	3.0	3.2	3.1	3.0	1.2	3.4	1.4	1.4	After death, from heart blood
44	-Almost pure culture	1 c.c. intravenously caused high fever, loss of weight, paralysis, and death	8-20-30, seldom more	3.4	2.3	3.1	3.4	0.9	2.1	1.0	1.1	2 days before death

TABLE 17

Beta Hemolytic Streptococci from Severe Cases of Scarlet Fever (Temperature 104 or More, Delirium, Ulceration of the Throat, Edema of the Neck, etc., "Septic Scarlet Fever")

No.	Hemolytic Colonies	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Carbohydrate Reactions								Day of Disease
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin	
8	Large numbers, 200–300	0.5 c.c. intraperitoneally killed mouse in 48 hours	Long chains, 50–100	3.7	3.1	2.5	3.1	1.3	3.9	1.2	1.3	3rd
10	Large numbers, 200–300	0.5 c.c. intraperitoneally killed mouse in 24 hours	Long chains, 50–100	3.7	2.6	3.0	3.4	1.2	2.7	1.3	1.2	6th
12	5-6 colonies	1 c.c. in rabbit caused high fever, loss of weight, cardiac involvement. Recovered	60–100	2.3	2.0	2.3	2.9	1.1	2.8	1.1	1.0	24th
20	Almost pure culture, 200–250	1 c.c. in rabbit caused high fever, loss of weight, joint-involvement, death in 6 wk.	40-50	2.5	3.1	3.6	3.4	1.2	3.8	1.4	1.5	4th
24	Almost pure culture	1 c.c. in rabbit caused high fever, joint-involvement. Recovery	10-20, seldom longer	2.1	2.2	2.1	2.2	2.3	1.9	1.0	1.3	4th to 5th
23	Almost pure culture	1 c.c. in rabbit caused high fever, marked loss of weight. Recovery	30-40	2.5	2.6	2.6	2.3	2.1	3.7	1.0	1.3	4th
25	Almost pure culture, 200	1 c.c. in rabbit caused high fever, joint lesions. Recovery	30-40	2.5	2.3	2.6	2.7	3.2	2.9	1.0	1.3	5th
32	Almost pure culture, 200	1 c.c. in rabbit caused high fever, joint lesions. Recovery	Chains very and interlacing	3.8	4.0	4.4	4.3	1.3	3.0	1.6	1.3	10th
41	Almost pure culture, 200–300	1 c.c. in rabbit killed in 48 hours	30-40 or longer	2.3	3.7	3.8	3.4	3.7	3.3	1.5	1.5	4th
46	40–50	0.5 c.c. in mouse killed in 1 mo. (purulent pericarditis)	8-10-20, seldom longer	3.6	2.9	2.7	2.6	1.1	2.8	1.4	1.4	9th. Almost recovered when culture was taken
53	15–20	0.5 c.c. in mouse killed in 72 hours	8–10, sometimes 20-30	2.5	2.8	3.1	3.2	1.5	3.0	1.5	1.5	7th. Much improved on day cuture was taken
70	Almost pure culture	1 c.c. intravenously in rabbit caused high fever, loss of weight. Recovery	Long chains, 50-80	2.0	2.2	2.1	2.2	1.2	2.4	1.2	1.3	8th. Still very septic

disease. In each instance, a typical beta hemolytic streptococcus was isolated from the throat, or from the blood, or both. Each streptococcus had high virulence for animals. These patients undoubtedly died from streptococcemia.

The Cases of Septic Scarlet Fever.—Table 17 includes these cases. Beta hemolytic streptococci were present in these throats in almost pure growth;

TABLE 18

BETA HEMOLYTIC STREPTOCOCCI FROM SCARLET FEVER OF MODERATE SEVERITY (TEMP. 102-103, EXUDATE IN THROAT BUT NO ULCERATION, MODERATE PROSTRATION)

No.	Hemolytic Colonies	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Carbohydrate Reactions								Day of Disease
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin	
5	40-50	0.5 c.c. killed mouse in 36 hours	Long and interlacing	3.9	3.3	3.0	2.4	1.3	3.3	1.2	1.4	3rd
9	Almost pure culture, 200	0.5 c.c. killed mouse in 4 days	40-50	4.0	3.8	4.0	4.1	3.8	4.1	1.3	1.4	3rd
13	200-300	0.5 c.c. killed mouse in 48 hours	Long and interlacing	2.6	3.4	3.0	3.1	1.5	1.4	1.2	1.7	4th
15	60-75	0.5 c.c. did not kill mouse	Long and interlacing	2.6	2.1	2.3	2.6	1.1	1.2	1.1	1.0	16th
17	4-5	0.5 c.c. did not kill mouse	30-40	2.4	2.1	2.3	2.3	2.2	2.2	1.1	1.1	2nd
22*	Almost pure culture, 150-200	0.5 c.c. killed mouse in 3 days	8-10, seldom more	2.1	2.2	3.0	2.4	1.0	2.3	1.2	1.3	7th
27	50-70	0.5 c.c. made mouse very ill. Recovered	2-4-8	2.8	2.9	2.4	3.1	1.6	1.1	1.3	1.1	10th
33	Almost pure culture, 150-200	1 c.c. in rabbit caused moderate fever, joint localization. Recovery	30-40	4.2	4.0	4.5	4.5	3.1	4.8	1.5	1.5	4th
52	4-5	0.5 c.c. killed mouse in 3 days	20-30	2.5	2.6	2.9	2.9	1.6	2.4	1.6	1.6	3rd
54	40-50	0.5 c.c. killed mouse in 48 hours	40-50	2.3	2.7	2.5	2.6	1.5	2.6	1.5	1.4	3rd
55	2-3	0.5 c.c. killed mouse in 36 hours	20-30-40	2.9	2.7	2.8	2.7	1.5	2.7	1.8	1.7	3rd
57	8-10	0.5 c.c. did not kill mouse	Long and interlacing	5.5	5.2	5.2	6.4	1.2	4.8	4.1	4.7	4th
66	4-5	0.5 c.c. killed mouse in 10 days	Long and interlacing	2.0	1.8	2.3	2.2	2.1	1.3	1.6	1.3	8th
69	6-7	1 c.c. killed rabbit in 6 days	50-60	2.6	2.3	2.4	3.0	1.2	2.3	1.1	1.3	9th

* This case almost belongs in the severe-scarlet-fever group.

and in very large numbers from the 3rd to the 6th day of the disease, disappearing rapidly as the child recovered. With one exception the strains had high virulence for animals. A curve of the relative numbers of beta hemolytic streptococci in the throat of the child would correspond closely to its temperature curve or to any other index indicating the severity of the disease.

The Cases of Moderate Severity.—These cases are included in Table 18. All the throats contained streptococci of the beta type, but in much smaller numbers than in the cases of septic scarlet fever. As the symptoms of the child improved, there was a corresponding decrease in the number of streptococci in the throat.

The Cases of Mild Scarlet Fever.—Table 19 summarizes the characteristics of the strains of streptococci isolated from the throats of mild cases of scarlet fever. In the throats of only 2 of the 8 cases was the typical beta hemolytic streptococcus found, and here in comparatively small numbers. The virulence for animals of all the isolated strains was low, either not killing the mice at all, or causing death in from 7 to 10 days.

Carriers.—The carrier cases, Group V, are of great interest. These are the most dangerous to the community. Case 47 is typical. After a severe attack of scarlet fever, this patient developed a double otitis media. At the end of 9 weeks the discharge finally ceased, and the child was sent home. Two days later the ears began to discharge again, and 4 days later 2 members of the child's family developed severe scarlet fever. A streptococcus with identical characteristics was isolated from the throats of the patients with scarlet fever and from the ears of the child with otitis media. It has become

TABLE 19

BETA HEMOLYTIC STREPTOCOCCI FROM MILD CASES OF SCARLET FEVER (TEMP. 99-101, NO EXUDATE IN THROAT, NO PROSTRATION)

No.	Hemolytic Colonies	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Carbohydrate Reactions								Day of Disease
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin	
16	Not typical beta	1 c.c. did not kill mouse	4-8-12	4.3	5.7	3.7	5.8	1.3	3.6	3.0	3.4	35th
25	Not typical beta	1 c.c. did not kill mouse	30-40	2.7	1.3	2.4	2.0	2.4	3.0	1.2	1.0	4-5th
51*	30-50	0.5 c.c. killed mouse in 9 days	8-10 to 20-25	2.5	2.5	2.7	2.6	2.6	2.4	1.6	1.7	3rd
56	Not typical beta. 2-3 colonies	0.5 c.c. killed mouse in 4 days	30-40	3.5	2.5	2.4	2.7	1.4	2.4	1.3	1.4	3rd
58	Not typical beta. 2-4 colonies	0.5 c.c. killed mouse in 1 month (purulent peritonitis)	4-8, some 20	2.6	2.6	2.8	2.5	1.4	2.8	1.3	1.2	6th
63	30-40 colonies	0.5 c.c. did not kill mouse	4-8-12, some 20-30	3.7	3.4	3.6	3.2	2.0	3.2	1.2	1.4	6th
65	2-3	0.5 c.c. killed mouse in 7 days	4-8-12, some 30-40	2.2	2.0	2.3	2.3	1.3	2.4	1.1	1.2	5th
71	8-12	0.5 c.c. did not kill mouse	20-30	3.2	3.0	3.2	2.3	1.7	4.4	1.4	1.4	8th

* This case was more severe than the others included in this group.

so well established that children who have otitis media as a complication of scarlet fever are probably carriers of the disease, that no modern hospital for contagious diseases allows the return of these children to community life until all foci of infection have been healed.

TABLE 20

BETA HEMOLYTIC STREPTOCOCCI FROM CARRIER CASES OF SCARLET FEVER

No.	Hemolytic Colonies	Virulence for Animals (24-hr. broth culture)	Length of Chain (cocci)	Carbohydrate Reactions								Day of Disease
				Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin	
8	12-14, nose	1 c.c. in rabbit caused high fever and joint-involvement. Recovery	40-50	2.4	2.5	2.1	2.2	1.1	3.1	0.9	1.0	48th
29	4-5, nose	0.5 c.c. intraperitoneally killed in 70 hours	Very long chains	2.6	2.1	2.9	2.2	1.1	2.7	1.2	1.0	56th
50	Pure culture, ears	0.5 c.c. killed mouse in 24 hours	20-30	3.2	2.7	2.4	3.0	1.4	2.6	1.3	1.4	34th
59	10-20, nose	0.5 c.c. killed mouse in 24 hours	8-12-16, seldom longer	2.4	2.7	2.7	2.3	1.0	2.5	1.4	1.4	129th
47	30-40, ears	0.5 c.c. killed mouse in 20 hours	25-30, no very long chain	2.2	3.2	2.4	2.3	1.4	2.3	1.4	1.6	70th
60	Ears none on original. Isolated from rabbit joint	0.5 c.c. killed mouse in 36 hours. 1 c.c. in rabbit gave high fever and joint-involvement	50-60	3.2	2.7	2.3	2.6	1.0	2.8	1.2	1.3	36th
61	Ears. None from original. Isolated from rabbit joint	1 c.c. in rabbit gave high fever, loss of weight, and joint-involvement	30-40	2.3	2.4	3.0	2.1	1.4	2.4	1.2	1.2	56th
62	300-350, ears	0.5 c.c. killed mouse in 36 hours (peritonitis and pericarditis)	40-50	2.4	2.0	2.3	2.2	1.0	2.1	1.4	1.5	16th
67	Ears. None from original. Isolated from joint of rabbit	1 c.c. in rabbit caused fever loss of weight, and joint-involvement	30-40	2.3	2.4	2.7	3.2	1.6	2.5	1.4	1.3	90th
68	70-80 colonies, ears	1 c.c. in rabbit caused high fever, loss of weight, and death in 5 days	20-30, some interlacing masses	2.3	2.4	2.0	2.4	1.0	2.8	1.1	1.0	18th

Ten cases of the carrier type were studied. A summary of the results is given in Table 20. In each of the ten cases, a typical beta hemolytic streptococcus was isolated from the purulent material. If the infection was a recent one, as in Cases 62 and 68, the streptococcus was present in large numbers

and could be isolated with ease. In some of the long standing cases, 60, 61, and 67, it was found impossible to isolate a hemolytic streptococcus by means of the blood-agar plate because of a large number of secondary contaminating organisms that rapidly overspread the plate. Animal inoculation was at first unsuccessful; a large amount of the suspension of the purulent discharge caused death of the rabbits, but as a rule the rapidly spreading organism was also present in the blood culture; a small amount produced no symptoms. The interesting work of Faber[18] offered a solution of the problem. He has shown that one can sensitize the joints of rabbits to various strains of streptococci.

Four rabbits were chosen that had had, 3 months previously, an intravenous injection of the beta hemolytic streptococcus. Each had developed the usual high temperature, with loss of weight and polyarthritis, but had finally recovered completely. Their blood still showed the presence of agglutinins for the beta hemolytic streptococcus. One 4-mm. loopful of the purulent material from each of Cases 60, 61, and 67, was added to 10 c.c. of salt solution and well shaken. The rabbits were injected with 0.5 c.c. each of this suspension. In from 4 to 6 days each rabbit developed an arthritis in the same joint that had been previously affected. Pure cultures of beta hemolytic streptococcus were isolated from the affected joints and studied.

Tho all the evidence seems to indicate that scarlet fever is not caused by the beta hemolytic streptococcus, nevertheless this strain plays a part in the course of the disease; in those cases that I have studied, the severity of the disease bears a close relation to the presence of, and also to the number of, the beta hemolytic streptococci in the throat. In rare instances a child develops "fulminating scarlet fever" and dies on the 2nd or 3rd day of the disease. This type of case probably succumbs to the actual etiologic agent of scarlet fever. Septic scarlet fever as seen in America, however, is, secondarily at least, a streptococcus infection, and in fatal scarlet fever, death is usually due to streptococcemia. These facts perhaps explain the results of Gabritschewsky[19] and others with a streptococcus vaccine as a prophylactic against scarlet fever.

It seems not improbable that the beta hemolytic strain, which has been shown to be so persistent in cases of septic sore throat, may also be the factor which causes the chronic purulent discharges that so frequently follow scarlet fever. These results would seem to explain how it is possible for epidemics of septic sore throat to be caused by a case of scarlet fever. The discharges from a case of scarlet fever may contaminate milk either with the actual etiologic agent of scarlet fever, or with the secondary invader, or with both. Thus, contaminated milk may produce mild scarlet fever, septic sore throat, or septic scarlet

[18] Jour. Exper. Med., 1915, 22, p. 615.
[19] Centralbl. f. Bakteriol., I. O., 1906, 41, p. 719.

fever, depending on whether the individual is infected with one or both of these factors.

In summary: The beta hemolytic streptococcus was found in large numbers and in almost pure growth in the throats of all the severe cases of scarlet fever. It was not found in the throats of all the moderate or mild cases of scarlet fever, and when found occurred in less numbers.

The severity of a case of scarlet fever is usually directly proportional to the virulence of the beta hemolytic streptococcus found in the throat.

The chronic purulent discharges from the nose or ears of a case of scarlet fever may contain the beta hemolytic streptococcus for from 5 to 6 months after acute symptoms of the disease have subsided.

These results explain how epidemics of septic sore throat may be due to contamination of the milk supply with discharges from a case of scarlet fever.

FINAL SUMMARY

The epidemics of septic sore throat of Dorchester and the K. boarding school illustrate the fact that a hemolytic streptococcus may be found in the throats of the patients and also in the contaminated milk that caused the epidemic. This streptococcus, which seems to be a human strain, has definite characteristics by which it may be readily distinguished.

Persons with normal tonsils and normal mucous membranes of the nasopharynx do not harbor it in their throats except in rare instances. This fact probably explains the relative infrequency of contamination of milk, for the flora from normal human throats undoubtedly finds its way in many instances into the daily milk supply.

The beta hemolytic streptococci may be found in the throat for a long time after acute symptoms of septic sore throat have subsided. Individuals who have chronic "irritation" of the throat after an acute attack of septic sore throat are particularly apt to harbor the streptococcus and are therefore particularly dangerous to the community.

Sporadic tonsillitis may be due to the same streptococcus as is found in cases of septic sore throat. Clinically, it is often impossible to distinguish a severe case of sporadic, from a case of epidemic, tonsillitis; in all probability they are often the same disease. It is most likely that septic sore throat is kept alive in the community by means of the sporadic cases, or by means of carriers. Indeed, it seems most

probable that each infection with the beta hemolytic streptococcus is really an integral part of some epidemic. The infection may occur as an outspoken epidemic, as illustrated by the Dorchester outbreak, or it may be the result of contact infection, the mechanism of which is shown in the case of the B. hospital. It seems probable that cases which occur in interepidemic times, if their epidemiology could be traced, would usually prove to be a residual of some previous epidemic, which may, in turn, kindle a fresh epidemic. Every individual who harbors the beta hemolytic streptococcus for a long or short period of time is a danger to the community, in so far as he comes into intimate personal contact with his fellow men. Scarlet fever, in the severe and in the moderate type, is frequently accompanied by an infection with the beta hemolytic streptococcus, and this organism may remain in the discharges from the ears or nose of the patient for several months.

Epidemic tonsillitis, then, differs from sporadic tonsillitis due to this streptococcus in that the latter is an infection of the individual, usually by direct contact, and the former is an infection of the whole community, usually by indirect contact.

Septic sore throat, or epidemic tonsillitis, is not, therefore, a disease due wholly to the use of contaminated milk, and it is not necessary that the disease be present in the community in epidemic form, in order to make a definite diagnosis. The diagnosis can readily be made by comparatively simple laboratory procedures, and within a short period of time. The disease does not spread from one community to another in great epidemic waves, as do smallpox and influenza; its mode of spread resembles that of diphtheria or scarlet fever, and, as with these two diseases, the etiologic factor is probably more or less present in most communities of any size at all times, either in sporadic cases of the disease, or in the throats of carriers. Fortunately the individual who harbors this streptococcus is not of special danger to the community unless he comes into intimate personal contact with some of the activities of the community, as handling the milk supply.

CONCLUSIONS

A streptococcus was isolated from the throats of patients of the Dorchester epidemic of septic sore throat that was identical in its cultural characteristics with a streptococcus isolated from the suspected milk.

A streptococcus was isolated from the throats of patients in the milk epidemic at the K. boarding school that was identical in cultural

characteristics and agglutination reactions with a streptococcus isolated from the udder of one of the cows from the school dairy.

The types of streptococci isolated from the Dorchester milk epidemic, and the K. boarding school epidemic, are identical in their cultural characteristics and virulence for animals with the type of streptococci described by Smith and Brown in their studies of the milk epidemics of Massachusetts of 1914-1915. This type of streptococcus is a human strain, with a group of definite characteristics clearly described by Smith and Brown.

The streptococcus of this type is seldom found in normal throats (1% of the cases studied).

The throats of individuals who have had tonsillitis may harbor the beta hemolytic streptococcus for 3 or more months after acute symptoms of the disease have subsided.

This streptococcus is found in large numbers in the throats of a ertain proportion of cases of sporadic tonsillitis (5 of 20 studied).

Epidemic tonsillitis, or septic sore throat, is, therefore, not a disease due wholly to the drinking of contaminated milk. The disease may exist not only in the epidemic, but in the sporadic form.

Tonsillitis, epidemic and sporadic, due to the streptococcus of the beta hemolytic type, may be severe or moderate, or even mild.

The beta streptococcus is frequently found in the throats of acute cases of scarlet fever, and may remain for months in the purulent discharges of the cases complicated by otitis media, etc.

These facts explain how the discharges from a case of scarlet fever may contaminate a milk supply and produce a milk-borne epidemic not of scarlet fever, but of septic sore throat.

TECHNIC

There is great diversity of opinion as to the proper media for study of streptococci. For the sake of uniformity and for comparative results we have followed closely the methods used by Smith and Brown.

Hemolysis.—Classification of types of hemolysis has been studied but little. The usual classification is either hemolysis, or no hemolysis, with occasional mention of incomplete hemolysis. The beta hemolytic streptococcus of Smith and Brown on horse-blood-agar plates forms a small lanceolate colony in the depths of the media. Around the colony is a clear-cut zone of hemolysis, from 2 to 4 mm. in diameter, at the end of from 24 to 48 hours. Under the low power of the microscope complete disappearance of the outline of

the red cells is noted. The border of the hemolytic zone is rather abruptly limited by the dense mass of red cells.

It is important to remember that the blood of different animals produces different types of hemolysis. Guinea-pig blood is particularly unsatisfactory. Dog blood produces a brownish discoloration. Washed corpuscles give a different type of hemolysis from that of whole blood, with narrower and less well-defined borders. Some of these peculiarities might be explained on the basis of acid-production by the organism. Cumming[19] has shown a great variation in the hemolysis of the blood of different animals when weak solutions of acid and alkali are added to the blood. Many of the factors in the production of hemolysis, however, are not understood.

For the hemolytic plates, I have used 10 c.c. of veal infusion agar, + 0.8 to + 1, using Witte's peptone. The American peptones have produced unsatisfactory results in these studies.

The amount of blood is also important. One cubic centimeter of defibrinated horse blood is added to each agar tube. The plate is read at the end of 48 hours' incubation. The deep, and not the surface, colonies are taken as the standard. Results are not read if more than 50 colonies develop on the plate, since overcrowding of colonies produces noncharacteristic zones.

My experience has been that under similar conditions, a given organism, if freshly isolated (within from 2 to 3 weeks), will produce a definite and uniform type of hemolysis, and that the diameter of the hemolytic zone will be constant within a moderate latitude. For example, an organism will not have a 2-mm. zone at one time and a 4-mm. zone at the next subcultivation. These hemolytic characteristics change, however, in some instances after prolonged cultivation on artificial media.

Sugar Reactions.—The sugar reactions were studied by the generally accepted standard methods. Sugar-free veal infusion broth was prepared in 9-c.c. amounts, brought to +1, and sterilized. The carbohydrates were sterilized separately in the autoclave at 10 pounds' pressure for 30 minutes in 10% solution, and 1 c.c. of the 10% sugar solution added to each 9 c.c. of broth. All tubes were incubated for 24 hours to test sterility.

The carbohydrates used were dextrose, lactose, maltose, saccharose, mannite, salicin, raffinose, and inulin. Each tube was inoculated with 1 loop from a 24-hour broth culture of the organism to be studied and incubated for 7 days. The readings are all of titrations while hot, phenolphthalein being used as an indicator. Results are reported according to the usual method; that is, the number of cubic centimeters of normal NaOH which would be necessary to neutralize 100 c.c. of the broth.

This method of determining the reactions is subject to criticism. Since the work was begun, there have appeared several studies on the buffer reactions of meat infusion, and the determination of its true acidity by means of the electrolyte (Clark,[20] Bouvie,[21] and others). I agree with the investigators that media should be standardized in relation to its hydrogen ionization rather than its titratable acidity. For the purposes of our study, however, the titration of the media has been just as satisfactory as the determination of the Ph.

In order to satisfy myself on this point, I chose 5 cultures of hemolytic streptococci from human throats and planted them in the sugar tubes. At the end of 7 days the tubes were titrated in the cold and then in the hot

[19] Jour. Infect. Dis., 1916, 18, p. 151.
[20] Ibid., 1915, 17, p. 109.
[21] Jour. Med. Research, 1915, 28, p. 295.

with phenolphthalein as the indicator. For titration in the work reported here freshly boiled distilled water was used. As the tables show, there is very little variation between the titrations in the hot and in the cold. The hydrogen-ion concentration was then determined in the same tubes, not by the electrolyte, but by the colorimetric method devised by Henderson, and used by Henderson and Palmer[22] and their associates in the determination of the hydrogen-ion concentration of the urine (see Table 21).

The correspondence of the hydrogen-ion concentration with the titratable acidity is very close. The numbers of course represent the logarithms of the hydrogen-ion concentration and are a convenient method of expression. Thus 5.1 represents a greater degree of acidity than 7. The hydrogen-ion concentration of the control uninoculated tube was 7.2, or about the hydrogen-ion concentration of blood, which is 7.4.

TABLE 21

COMPARISON OF THE DETERMINATIONS OF ACIDITY IN MEDIA (ALL CULTURES ARE STREPTOCOCCI FROM HUMAN THROATS)

No.	Type of Hemolysis	Virulence for Animals (24-hr. broth culture)	Method of Titration	Sugar Reactions: Dextrose	Lactose	Maltose	Saccharose	Mannite	Salicin	Raffinose	Inulin
56	Beta	0.5 c.c. killed mouse in 7 days	Titration cold......	3.3	3.2	2.1	2.4	1.2	2.3	1.3	1.3
			Titration hot.......	3.5	3.5	2.4	2.7	1.3	2.4	1.5	1.6
			PH concentration...	5.1	5.5	5.4	5.5	7.2	5.7	7.0	7.2
57	Not typical beta. Incomplete	0.5 c.c. did not kill mouse	Titration cold......	5.5	5.5	5.3	6.7	1.2	4.8	4.1	4.7
			Titration hot.......	5.6	5.7	5.4	6.8	1.3	5.0	4.2	4.9
			PH concentration...	4.5	4.4	4.6	4.4	7.4	4.7	4.8	4.7
58	Beta	0.5 c.c. killed mouse in 6 days	Titration cold......	2.4	2.4	2.5	2.4	1.4	2.6	1.3	1.3
			Titration hot.......	2.6	2.6	2.7	2.5	1.5	2.8	1.4	1.5
			PH concentration..	5.4	5.5	5.6	5.6	7.2	5.5	7.2	7.0
59	Beta	0.5 c.c. killed mouse in 3 days	Titration cold......	2.2	2.5	2.6	2.0	1.4	2.1	1.5	1.5
			Titration hot.......	2.3	2.7	2.7	2.3	1.6	2.5	1.7	1.7
			PH concentration..	5.7	5.5	5.6	5.8	7.2	5.7	7.2	7.2
60	Beta	0.5 c.c. killed mouse in 2 days	Titration cold......	2.4	2.4	2.7	2.5	1.3	2.3	1.5	1.4
			Titration hot.......	2.5	2.6	2.9	2.7	1.5	2.4	1.8	1.6
			PH concentration..	5.4	5.5	5.3	5.4	7.0	5.6	7.0	7.0

Streptococci were planted in the sugar broths and incubated for 7 days. Each tube was titrated in the cold and then in the hot, phenolphthalein being used as indicator. The hydrogen ionization concentration of the same tube was then determined by the Henderson method.

Another point in the titration of media has caused no little discussion; namely, the amount of acidity which must be produced in a tube to indicate whether or not an organism splits a certain sugar. The streptococci which I have studied, if they have the ability to ferment a carbohydrate, do so in a definite and clear-cut way. The acidity in those tubes in which the sugar has been split is remarkably constant. On the other hand, the sugars which have not been acted upon give uniform end reactions. This fact has been more clearly brought out by determination of the hydrogen-ion concentration. Thus, for example, an organism that ferments both dextrose and salicin will not ferment dextrose to a hydrogen-ion concentration of 4.8, and salicin to 5.8, but both to about the same acidity. This suggests that a streptococcus will

[22] Jour. Biol. Chem., 1912, 13, p. 363.

ferment a sugar until a hydrogen-ion concentration is reached which is incompatible with further activity of the organism. I have found as a general rule that the more pathogenic streptococci produce a less high acidity than the less pathogenic streptococci.

The Henderson colorimetric method for the determination of hydrogen-ion concentration is so simple, so accurate, and once the standard solutions are in stock, requires so little time, that I have found this method of determining the acidity of media a very practical one.

Virulence for Animals.—The virulence of the streptococci isolated from septic sore throat has been studied in many of the reported epidemics. The most complete study is that of the Chicago epidemic by Jackson.[15] The author produced severe polyarthritis in all the rabbits reported. The exact technic is not given. Smith and Brown injected rabbits with 1 c.c. of a 24-hour broth culture intravenously. We have followed this method. Temperature and weight charts were kept of each animal. This procedure enabled us to pick out the mildly pathogenic organisms, for in some instances the only reaction was an increased temperature for 3 or 4 days. It also made it possible to prognosticate joint-involvement, for a rise in temperature usually preceded the tenderness and swelling in each fresh joint.

When mice were used, 0.5 c.c. of a 24-hour broth culture was injected intraperitoneally. An organism is not called pathogenic for mice unless the mouse died, and the streptococcus was recovered from the peritoneum or heart blood. The pathogenicity for mice of 0.5 c.c. of a 24-hour broth culture intraperitoneally is a fair index of its pathogenicity in rabbits. Comparative results proved that in all the cases studied, a streptococcus which killed a mouse in from 3 to 4 days would produce high fever and marked loss of weight in rabbits, followed usually by polyarthritis, when 1 c.c. of a 24-hour broth was injected intravenously. For the preliminary studies mice were often used. In substantiation of the more important cultures rabbits were used for confirmatory evidence.

Capsule-Formation.— Previous reports, particularly those of Davis and Rosenow,[8] emphasize the importance of the capsule as a distinguishing characteristic of the streptococcus isolated from milk epidemics. This characteristic is not emphasized by Smith and Brown. The capsule of this type of streptococcus is not a true capsule but a capsular substance. All the beta hemolytic streptococci studied by us when freshly isolated have had a capsular substance, in some instances very little, in others more, in all cases disappearing rapidly on cultivation. Capsular substance, therefore, and also length of chain, are so variable and of so little differential importance that they are not reported in these studies.

Agglutination.—Twenty-four-hour broth cultures were shaken in a mechanical shaker for 15 minutes, then allowed to stand for 1 hour. The living suspension was added to the serum dilutions in small test tubes, the total fluid in each tube being 2 c.c. The tubes were placed in the incubator at 37.5 C. for 1 hour, then placed in the refrigerator and read at the end of 24 hours. C indicates complete clumping and sedimentation; ++++, complete clumping without complete sedimentation; +++, clumping nearly complete; ++, partial clumping; +, slight; ±, doubtful, and —, no clumping.